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Heavy Metals (Pb, Cd and Hg) Analysis in *Cheumatopsyche* sp. (Hydropsychidae, Trichoptera) as Bioindicator for Support Water Quality Assessment

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ABSTRACT

In this study, we utilized the larvae of *Cheumatopsyche* sp. (Insecta, Trichoptera, Hydropsychidae) as a key component to assess heavy metal contamination in the waters of the Bone River, Gorontalo, Indonesia. To measure the concentrations of heavy metals, specifically lead (Pb), cadmium (Cd), and mercury (Hg), an atomic absorption spectroscopy (AAS) was employed on both river sediments and the entire body of the *Cheumatopsyche* sp. larvae. Additionally,analysis was conducted using the SEM-EDX method to confirm the absorption of heavy metals in the larvae, particularly in the digestive tract. Sediment and larvae samples were collected from five sampling points along the main river, including reference sites in the upstream area. Our findings revealed that Hg and Cd levels were lower in the sediment compared to those in the larvae, while Pb levels were higher in the sediment. Examination of heavy metals in the digestive tract showed morphological abnormalities in larvae from the most contaminated sites. This research confirmed that *Cheumatopsyche* sp. larvae effectively absorb heavy metals, particularly Hg, validating their use as bioindicators in heavy metal bioassays.

INTRODUCTION

Anthropogenic activities mainly cause water heavy metal pollution **(Wang *et al.*, 2007; Liu *et al.*, 2018)**. Heavy metals are the most dangerous pollutants due to their toxic, carcinogenic, biomagnifying, and bio-accumulative characteristics. Many heavy metals accumulate in sediments, while the bottom substrate of waters serves as a habitat for macroinvertebrates and, therefore, has the potential to be contaminated with heavy metals. Macroinvertebrate communities generally integrate environmental changes in their habitat's physicochemical and ecological characteristics **(Rahayu *et al.*, 2015; Everall *et al.*, 2019)**. Alterations in the community composition, as well as the presence or absence of specific constituents at a particular site, can serve as indicators of the ecological well-being of freshwater **(Hart *et al.*, 2001; Benmatouk *et al.*, 2024)**. The sensitivity and response to various contaminants and the living characteristics of the macroinvertebrate community have great potential to be developed as a universal pollutant monitoring tool, especially in rivers. However, there is still a restricted quantity of bioindicators available for assessing heavy metal pollution.

The presence of heavy metals in water will accumulate in the tissues, potentially resulting in physiological disorders **(Kadim & Arfiati, 2022)**. The response of macroinvertebrates to heavy metals can be more complex than solely considering whether they are present or absent at the sampling location or the concentration of heavy metals within their bodies. Often these changes are not acute, but chronic and subtler, resulting in impaired growth affecting metabolism **(Bere *et al.*, 2016)**. Some macroinvertebrates can survive in water and accumulate different metals, thus reflecting continuous environmental degradation **(Girgin *et al.*, 2010; Chiba *et al.*, 2011)**. Aquatic insects tolerate and accumulate metals to varying degrees **(Prommi & Payakka, 2018)**. Aquatic invertebrates can assimilate metals into their bodies through external body surfaces or digestive pathways **(Rainbow *et al.*, 2015)**. **Wang (2002)**, in his study, stated that food particles are the main source of metals. The digestive tract of aquatic insect larvae generally has a tube-like shape extending to the anus **(Morgan *et al.*, 2002)**. Observing the internal organs of an organism, for example, the digestive tract is expected to provide information that heavy metals are absorbed in the body.

Tissue damage due to the accumulation of heavy metals causes cell damage, thus potentially interfering with their survival. The response of aquatic insects to heavy metals can be more complex than just assessing at the presence or concentration of heavy metals within the biota's body. Often the effects are not acute but chronic and less noticeable. Comprehending the biological response of organisms to heavy metal contamination at the cellular level holds great importantance to prove the link between the cause and effect of functional disturbances in aquatic insects.

Sampling of benthic invertebrates and species identification are both difficult and require the involvement of many specialist groups **(Tszydel *et al.*, 2015)**. In this study, the decision was made to utilize the presence of specific organisms that can effectively represent a particular stream or habitat type. Hydropschydae, in general, has begun to be used as a candidate bioindicator for determining heavy metal concentrations in rivers **(Sudarso, 2009)**. Several studies have reported using this family to monitor pollutants, especially heavy metals **(Sudarso & Yoga, 2015; Tszydel *et al.*, 2021; Rubio-Gracia *et al.*, 2022)**. In our previous study, we observed an association between heavy metal contamination in sediment samples and tissues of Hydropsychidae larvae. The genus *Cheumatopsyche* was chosen as an indicator organism to be investigated for the concentration of heavy metal content in its body because it was found to be distributed from upstream to downstream of the Bone River **(Kadim *et al.*, 2022a)** in conditions contaminated with Pb, Cd, and Hg **(Kadim *et al.*, 2022b)**.

Before employing *Cheumatopsyche* sp. as a bioindicator, it is essential to ascertain whether these larvae indeed accumulate heavy metals within their bodies. Apart from using the AAS (Atomic absorption spectrometry) method to determine the concentration of heavy metals in the entire body of the larvae, we also attempted using SEM-EDX analysis to identify trace elements in the digestive tract of *Cheumatopsyche* sp. larvae. Previously this method was used and recommended by **Tszydel *et al.* (2021)** to observe possible morphological deviations in the anal papillae of Hydropsychidae, *Hydropsyche angustipennis* larvae contaminated with heavy metals. Our objective was to evaluate the extent of heavy metal contamination and demonstrate the capability of these larvae to absorb heavy metals, thereby establishing their utility as bioindicators, particularly in river ecosystems. This analysis was carried out to obtain a description of the morphology, composition, and concentration distribution of heavy metal elements so that it can be seen whether there is a qualitative correlation between heavy metals in river bodies and those in the bodies of macroinvertebrates. The expectation is that the results of the SEM-EDX analysis will reveal and prove the presence of heavy metals absorbed in the bodies of macroinvertebrates, enabling the expression of heavy metal contamination in the Bone River, Gorontalo.

MATERIALS AND METHODS

1. Study area

The research was conducted on the mainstream Bone River, Gorontalo, Indonesia. The primary sources of anthropogenic activities near the Bone River's vicinity include residential areas, farming, industrial operations, sand extraction, and gold mining, all of which pose the risk of generating heavy metal contamination. Based on the results of water quality analysis, especially in the middle downstream area extending to the mouth of the Bone River, **Pateda *et al.* (2017)**, **Koniyo (2020)** reported that the Bone River had indications of heavy metal pollution. In addition, **Lihawa and Mahmud (2012)** reported that mercury levels were high in water and sediments in the tributaries of the Bone watershed, where gold tailings activity was present. Sampling was carried out at five observation stations selected based on anthropological factors that have the potential to become a source of metal contamination so that the analysis results are expected to show different levels of metals (Fig. 1). Furthermore, the potential existence of Hydropschidae larvae is taken into account when determining the choice of sampling sites. One of the 5 sampling stations in the Bone River's upstream area is located in Pinogu Village and is used as a reference site.

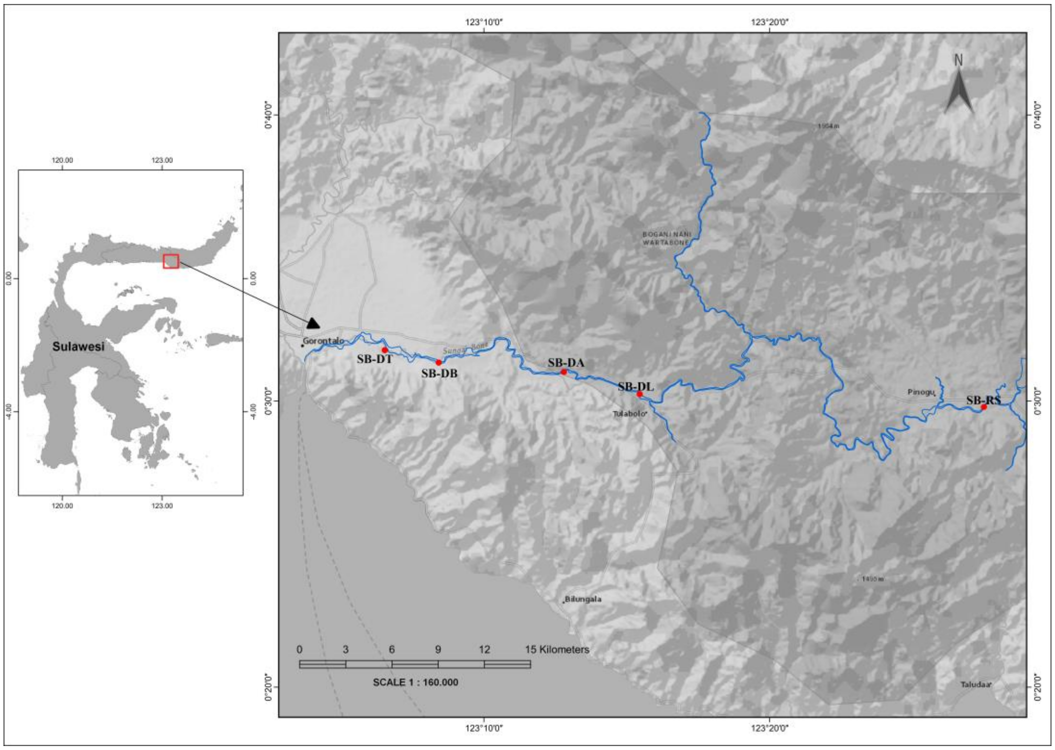

Fig. 1. Location of the Bone River. The red dot (•) indicates the sampling area

The coding of sampling locations is derived from the initials of the river name (Bone River-SB) and the area or village name (for example, Lombongo Village-DL). The first observation point was in the upstream region of the Bone River, located in Pinogu Village with a low level of anthropogenic activity, and was used as a reference site (control site). Observation point 1 (SB-RS) is geographically located at 0°29'48.07"N 123°27'30.57"E; observation point 2 (SB-DL) is geographically located at 0°31'0.25"N 123°11'46.59"E; observation point 3 (SB-DA) is geographically located at 0°32'0.99"N 123°10'20.59"E; observation point 4 (SB-DB) is geographically located at 0°31'37.63"N 123°7'38.89"E and observation point 5 (SB-DT) is geographically located at 0°31'48.83"N 123°6'33.66"E.

2. Heavy metal in Sediment and larvae of *Cheumatopsyche* sp.

Sampling took place between April and June 2022, a period characterized by minimal rainfall and relatively low river water discharge. A surface sediment sampling used a core with a depth of ± 10cm as much as ± 250g from each point **(Widiastuti *et al.*, 2019)**. Larval samples were taken using the kicking technique using hand nets with dimensions of 200 to 400mm wide, 2-3m high, 100-200mm shoulders (reinforcement) and 500µm eye size with a maximum reach distance of 10m. Hydropschidae larvae were manually collected by carefully inspecting the rocks within a maximum radius of 100 meters from the sampling site. Sampling was deemed complete once an adequate number of individuals were gathered to create three replicates for each area. These sorted larvae were then placed in 10ml capacity bottles and preserved using a 4% formaldehyde solution. Sediment and biota samples were prepared, stored in a cool box, and sent to the laboratory for analysis. Larvae of *Cheumatopsyche* sp. were sorted and then identified using a binocular microscope with 10-45 times magnification. Pb, Cd, and Hg concentrations in sediment and larval tissue were measured using an atomic absorption spectrophotometer (AAS).

3. Preparation of *Cheumatopsyche* sp. larvae. for histology and SEM-EDX observations

Only the larvae of *Cheumatopsyche* sp. with a 1-1.5cm length were used for histology and SEM-EDX analysis. The size determination is based on the assumption that at this stage, the larval samples have typically reached the 5th instar **(Zhang & Zhou, 2021)** and have undergone their final molting. This step is crucial to minimize the potential influence of molting on observational results, given the possibility of metal decomposition processes occurring within the body during molting at each stage of the larval instar phase **(Boggs, 1994; Tzsydel, 2021)**.

At this study stage, observations were made on the digestive tract of the larvae so that only the abdominal segments 1 to 9 of the larvae of *Cheumatopsyche* sp. were taken for analysis purposes. Previously paraffinized samples were cut crosswise using a microtome with a thickness of 5-6 microns. Some tissue sections were prepared for the Hematoxylin-Eosin (HE) staining procedure for observing tissue histology (procedure refers to **Yoga (2014)**) and the rest were prepared for the SEM-EDX procedure.

4. SEM-EDX procedure for *Cheumatopsyche* sp. larvae

The sectioning results were deparaffinized for SEM-EDX observation. Moreover, we carried out SEM-EDX analysis to compare the element composition on the surface of the larval body with digestion. The observation procedure using the SEM-EDX method on the larvae of the Hydropschidae family refers to the method of **Boggs (1994)**, **Jastrow (1998)** and **Tzsydel *et al*. (2021)**. The preparation procedure was as follows: Samples of larval abdominal tissue slices and body were fixed using 2.5% glutaraldehyde at pH 7.2 for 90 minutes then washed and post-fixed in 1.5% osmium tetraoxide pH 7.2 for 40 minutes, then the samples were placed on the cover glass to dry. Furthermore, the sample was washed with distilled water for 10 minutes and dehydrated for 10 minutes in acetone 30, 50, 70, 90, 95, 96, and 99%, at this stage, the specimen should not be contaminated with air. To remove water from the sample and avoid deformation, the preparation was dried with a critical point dryer at 73.8 bar with a temperature of 31^{o}C.

The sample was coated with carbon and gold in a vacuum evaporator for microscopy examination. The specimens were then attached to the pin with double-sided adhesive carbon tape, keeping their position parallel to the surface of the pin. The estimated abdominal thickness at the analysis point was only 1mm, which did not limit the SEM-EDX analysis or cause analysis errors **(Tzsydel *et al*., 2021)**. SEM-EDX Hitachi Tabletop

Microscope TM3000 and EDX INSPECT S50 performed an elemental analysis. The enumeration was carried out at three different measurement points.

5. Data analysis

The heavy metal data from sediment samples were evaluated using various regulations from several countries, as mentioned in Table (1). This was done because, as far as our research goes, we haven't found any rules from the Indonesian government regarding heavy metal standards, particularly in sediment. Meanwhile, the concentration of heavy metals in larval tissues was analyzed using the bioconcentration factor (BCF) (Table 1). BCF was measured to analyze the ability of the larvae *Cheumatopsyche* sp. to accumulate heavy metals Pb, Cd, and Hg from the surroundings into the tissue. Data were analyzed descriptively and expressed as mean ± SD for statistical tests. Analysis was performed using SPSS for Windows (SPSS version 22). The graph was performed using GraphPad Prism 9. The results of observations using the SEM-EDX microscope were analyzed descriptively and are expected to prove that heavy metals are absorbed in the bodies of *Cheumatopsyche* sp. larvae.

Tabel 1. Legal concentration limits of Pb, Cd, Hg in sediment and biota to various regulation and BCF category

Element	Regulation				BCF[e]	
	Sediment (ppm)			Biota (ppm)		
	ANZECC[a]	OSQG-LEL[b]	CCME-TEL[c]	Indonesian Quality Limit[d]	range	Category
Pb	50	31	31.2	0.2	>1000	Very high
Cd	1.5	0.6	0.7	0.1	100-1000	High
Hg	0.15	0.2	0.13	0.5	30-100	Moderate
					<30	Low

[a]Australian and New Zealand Environment and Conservation Council **(Simpson *et al.*, 2013)**; [b]Ontario Sediment Quality Guidelines-Lowest Effect Level; [c]Canadian Council of Ministers of the Environment-Threshold Effect Level **(Edward, 2020)**; [d]Indonesian Food and Drug Authority or BPOM; [e]**Sitorus *et al.* (2020)** and **Pebriani *et al.* (2022)**

RESULTS

1. Heavy metal in sediment

The sediment concentrations of heavy metals (Pb, Cd, and Hg) observed at the five stations can be seen in Fig. (2). Pb concentration was higher than the concentrations of Cd and Hg. Based on the measurement results, it is known that for heavy metal concentrations in sediments, the highest Hg and Cd were found in SB-DT with values of 0.25 ± 0.012 and 0.17 ± 0.011ppm, respectively. The highest Pb concentration was found in SB-DA with a value of 43.9 ± 1.5ppm. SB-RS exhibited the lowest concentrations of both lead (Pb), cadmium (Cd), and mercury (Hg). The statistical results of ANOVA (P<0.05) showed a very significant difference in the Pb, Cd, and Hg concentration between locations.

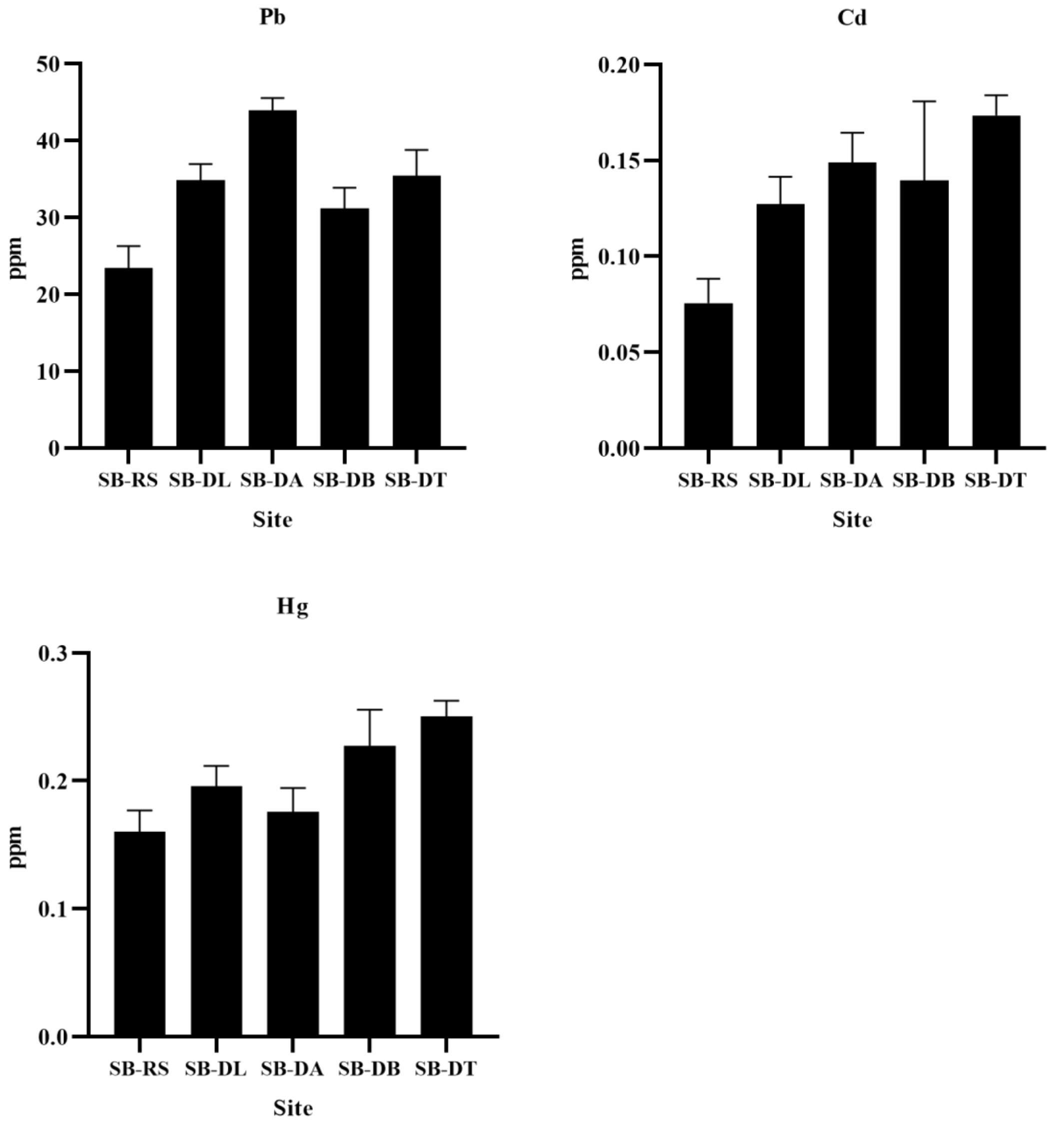

Fig. 2. Heavy metals level (Pb, Cd, and Hg) in the sediment sample

The results showed that the concentration of Pb in SB-DL; SB-DA; SB-DB; and SB-DT in this study was higher than the OSQG LEL and CCME TEL quality standard values but still did not surpass the standard values set by ANZECC. The Hg concentration exceeds the ANZECC and CCME TEL quality standard values (at all stations) and

OSQG LEL (at SB-DB and SB-DT). Meanwhile, the Cd concentration still meets the quality standards of these three standards.

2. Heavy metal in larvae of *Cheumatopsyche* sp.

The heavy metal content (Pb, Hg, and Cd) in the larvae observed from each sampling location is shown in Fig. (3). These values are then compared with the quality standards set by BPOM Regulation No. 5 of 2018. Data indicate that heavy metals Pb, Cd, and Hg accumulate in the bodies of *Cheumatopsyche* sp. The highest heavy metal values in the bodies of larvae all were obtained in SB-DA, with an Hg concentration of 0.609ppm, followed by Pb (0.526ppm) and Cd (0.374ppm). The larvae of *Cheumatopsyche* sp. accumulated lower metals in less contaminated areas (SB-RS) and higher in SB-DA, then decreased in SB-DB and increased again in SB-DT. The results of ANOVA statistic ($P<0.05$) showed a significant difference across locations with Pb, Cd, and Hg concentration within larvae body.

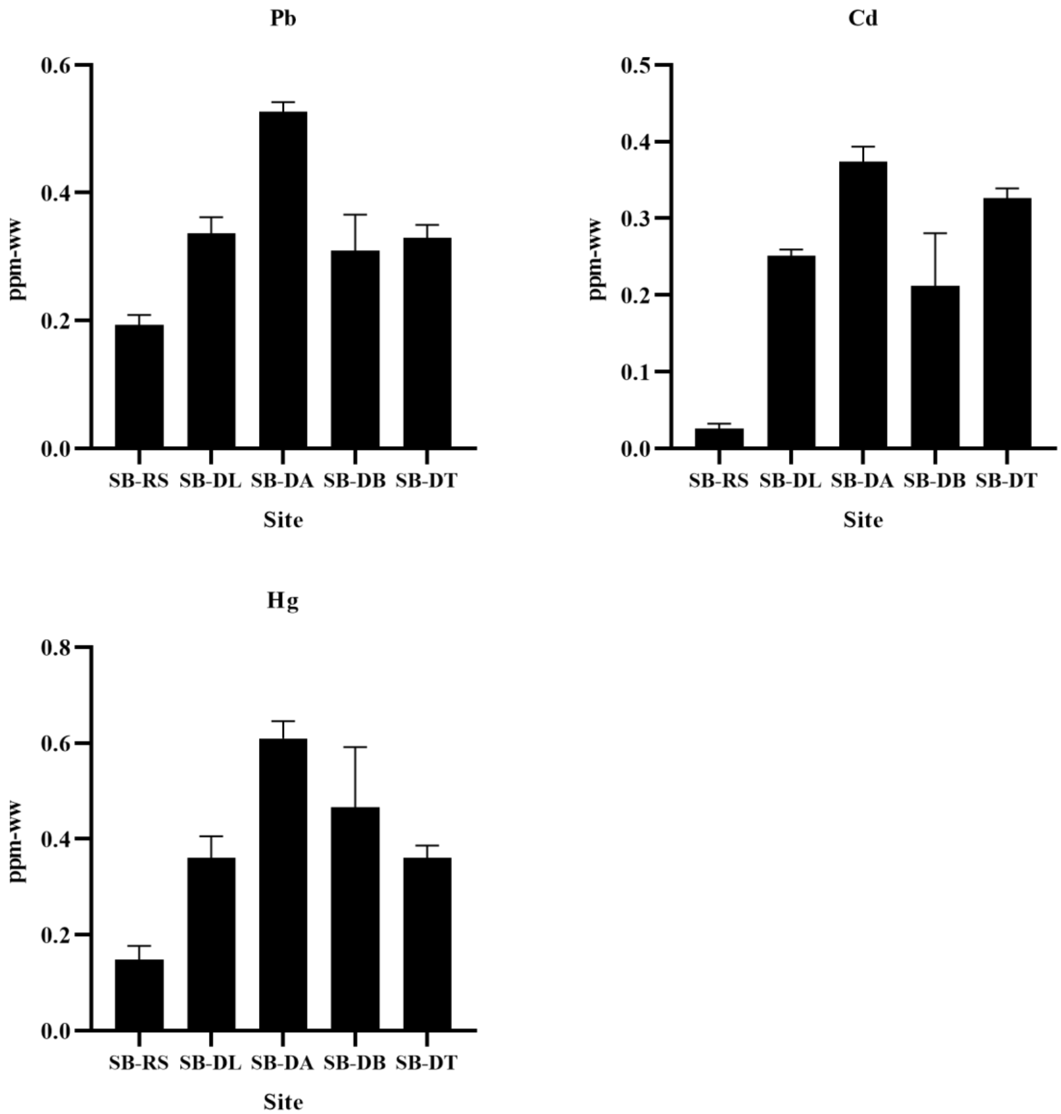

Fig. 3. Heavy metals level (Pb, Cd, and Hg) in whole-body of the larvae of *Cheumatopsyche* sp.

In this study, the calculation of the bioconcentration factor (BCF) was also carried out (Table 2). BCF value is a useful metric for expressing the degree of bio-concentration. It is calculated by comparing the concentration of a toxin in an organism to the concentration of the same toxin in the surrounding environment, resulting in a ratio **(Oost *et al.*, 2003)**. The higher the ratio, the more intense the toxin bio-concentration, in this case, the higher the metal content in the biota, the higher the BCF value, and the higher the organisms that accumulate heavy metals **(Crookes & Brooke, 2011)**. The calculation results show that the larvae of *Cheumatopsyche* sp. have an average bio-concentration of Pb<1 in each sampling location, while the average bio-concentration of Hg and Cd is >1. Biota with a value of BCF> 1 can be considered a bio-accumulator **(Takarina & Pin,**

2017; Sembel *et al.*, 2022). The BCF calculation results show that Hg has a higher bioconcentration factor value. This shows that the larvae of *Cheumatopsyche* sp. are more effective at accumulating Hg and Cd than Pb. The BCF value indicates that the larvae of *Cheumatopsyche* sp. are included in the low accumulative category (BCF <30).

Table 2. Bio-concentration factor in larvae of *Cheumatopsyche* sp.

Stasiun	BCF		
	Pb	Cd	Hg
SB-RS	0.0083	0.326	0.927
SB-DL	0.0097	1.971	1.838
SB-DA	0.0120	2.510	3.471
SB-DB	0.0099	1.518	2.053
SB-DT	0.0093	1.883	1.438
mean	0.010	1.642	1.945

3. Description of heavy metals in larvae body of *Cheumatopsyche* sp.

Further confirmation of heavy metals adsorbed in the larvae was confirmed by observing morphological abnormalities, using a SEM-EDX microscope, for the larvae depicted in the stomach tissue's cross sections (Fig. 4). Observing intestinal surface morphology abnormalities of *Cheumatopsyche* sp., based on cross-sectional histology with hematoxylin-eosin staining, was also compared.

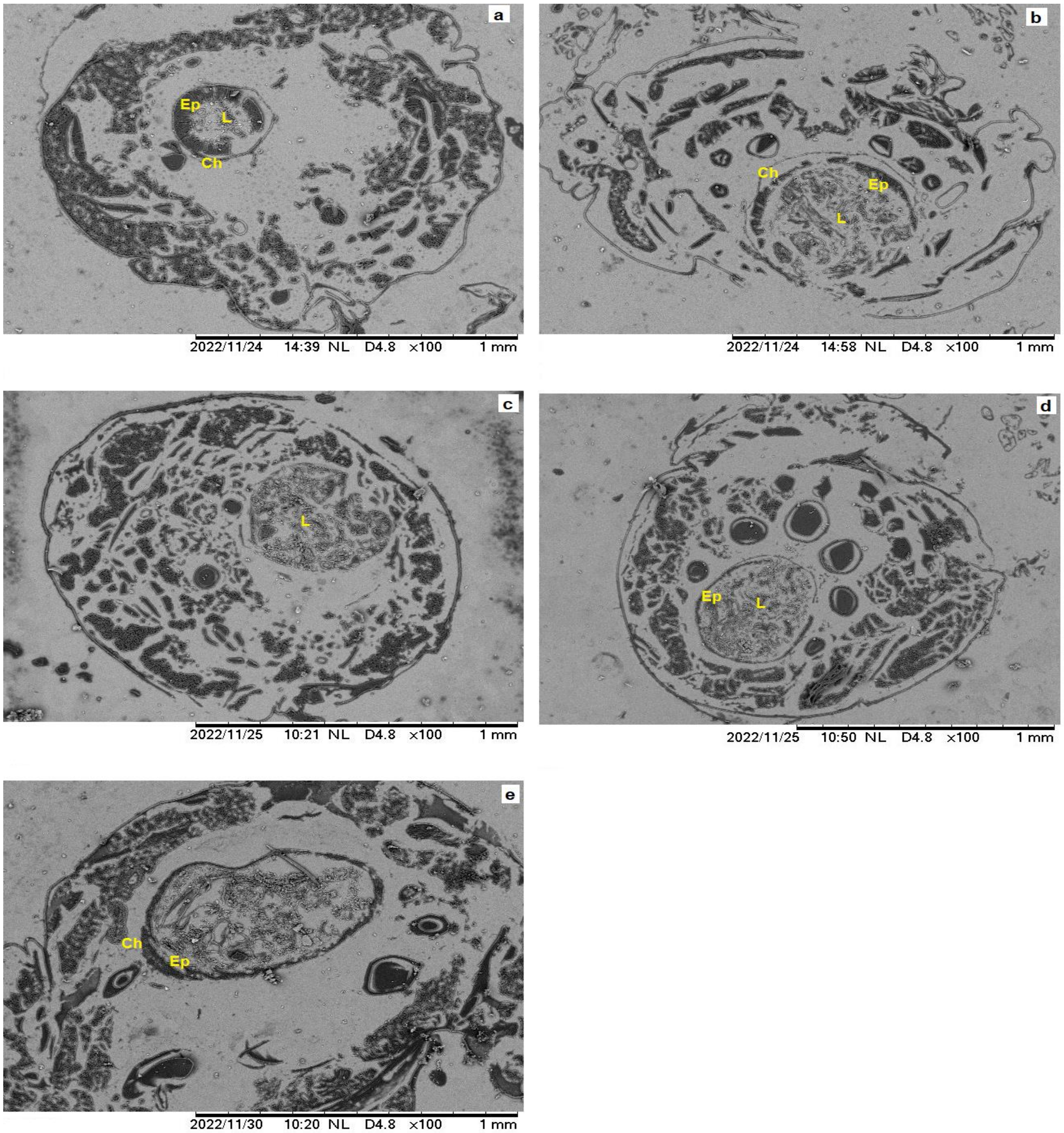

Fig. 4. Appearance of a tissue section from the stomach of *Cheumatopsyche* sp. transversely at each sampling station. **(a)** SB-RS sample; **(b)** SB-DL samples; **(c)** SB-DA samples; **(d)** SB-DB sample; (e) SB-DT samples. Ep = epithelial cells, Ch = chloragogen cells, L = lumen (SEM microscope with 100× magnification)

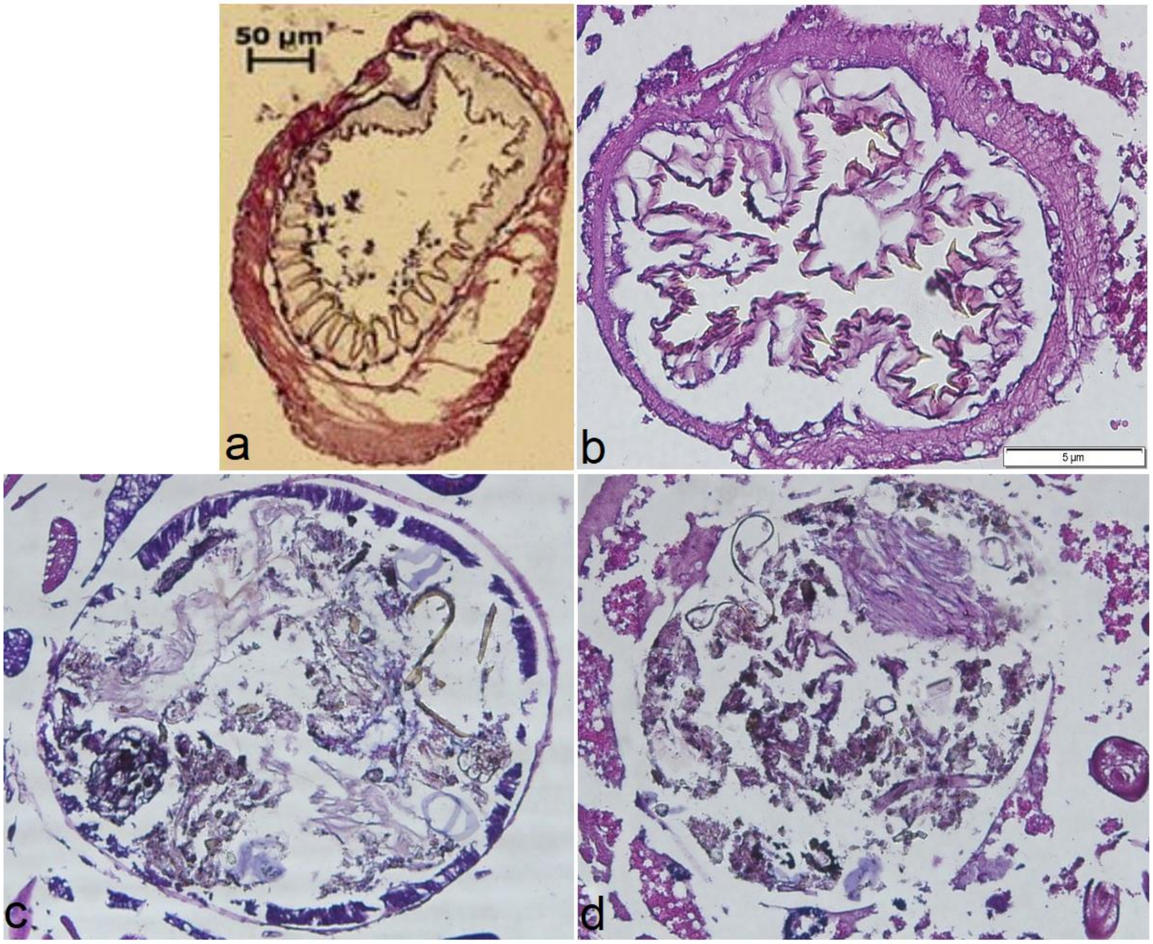

Fig. 5. Histological description of the cross-sectional surface of the lumen larvae of *Cheumatopsyche* sp. hematoxylin-eosin staining (10x magnification). Intestinal lumen cross-section of larvae **(a)** normal trichoptera **(Yoga, 2014)**; **(b)** normal, SB-RS sample; **(c and d)** with morphological abnormalities.

According to **Yoga (2014)**, the digestive tract of *Cheumatopsyche* larvae has large and smaller epithelial cells. These cells function to secrete digestive enzymes and store or bind contaminants, one of which is heavy metals. In his research on the histology of the digestive tract larvae of *Cheumatopsyche* sp., he explained that in parts of the intestine that are not contaminated with heavy metals, the epithelial cells will still appear thicker because they contain lipids. The thickness of the epithelial cells will shrink as contamination increases, and ultimately, the lumen diameter will widen. In this study, the results of photographs using an SEM microscope (Fig. 4) supported by histology with HE staining (Fig. 5) showed a similar appearance. Larval samples were taken from the reference site (SB-RS), where conditions of light heavy metal contamination led to a small lumen diameter with epithelial cells that were still thick and contained lipids. Alterations in lumen area were observed in samples from four additional locations where heavy metal concentrations, whether in water, sediment, or larval tissue, were elevated when compared to the reference site. SEM-EDX analysis of the body and lumen revealed the presence of 11 elements. The dominance of elements in larvae is presented in the highest to lowest order C>O>Pt>Na>Zn>Zr>Si>Hg>Pb>S>Cd. At the same time, the lumen tissue only shows the presence of 6 elements in the highest to lowest order, namely O>C >Pt>Hg>Pb>S (Table 3).

Table 3. The mean value of elements (Wt% - weight ratio) accumulated in larvae of *Cheumatopsyche* sp.

Site		*Wt* %										
		C	O	Cd	Hg	Pb	Pt	Zr	Zn	Na	S	Si
SB-RS	B	54,66	40,76	1,009	0,371	0,426	-	1,16	-	4,60	-	-
	L	36,43	41,39	-	6,415	2,691	13,08	-	-	-	-	-
SB-DL	B	56,05	32,92	-	0,554	0,606	7,19	4,74	4,40	-	0,89	3,68
	L	42,86	36,28	-	6,259	2,623	11,98	-	-	-	-	-
SB-DA	B	54,31	38,52	0,015	1,061	0,595	8,77	2,23	-	-	-	-
	L	46,55	36,89	-	5,355	2,198	9,02	-	-	-	-	-
SB-DB	B	45,55	49,00	0,024	0,750	0,457	4,24	-	-	-	-	-
	L	38,06	43,73	-	5,482	2,452	9,90	-	-	-	0,37	-
SB-DT	B	52,30	37,52	0,082	1,369	0,899	5,66	1,99	-	6,75	0,20	0,27
	L	34,18	46,99	-	5,793	2,702	10,34	-	-	-	-	-

B= body; L= lumen

Table (3) shows that the lowest values for Pb and Hg elements in the larvae body are at the reference site (SB-RS). Hg reached a maximum weight (1.369%) in the body of larvae collected at the SB-DT sampling site and 6.415% (lumen) at reference sites. Hg dominates over Pb and Cd in the larval body, SB-DA and SB-DB, and all lumen areas. Pb and Hg were detected in larvae in all sampling locations (highest value of Pb in SB-DT), while Cd was the only element not detected in the body parts of samples in SB-DL. Cd was not detected in the lumen at all observation stations. Cd reached a maximum value (1.009%) in the body of larvae collected at the reference site (SB-RS).

DISCUSSION

Sediment has the capacity to retain over 99% of the heavy metals that are introduced into the water **(Salomons, 1995)**. River sediments are carriers of heavy metals and thus play an important role in assessing and tracking contamination sources. In this study, the concentrations of Pb and Cd metals in sediments increased from the SB-RS area to SB-DA, and then the gradient decreased in SB-DB while increasing again in SB-DT. Meanwhile, the concentration gradient for Hg tends to increase downstream (SB-DT). These findings indicate that the relative proportions of metals across all sampling locations remain consistent. Consequently, differences in the concentration of heavy metals in the sediments may be attributed to contamination variations. The measurement results also showed that the concentrations of Pb and Cd (except at the SB-RS site) which accumulated in the bodies of *Cheumatopsyche* sp. exceeded the limits set by BPOM Regulation No. 5 of 2018. Meanwhile, only the Hg concentration of larval samples in SB-DA exceeded the specified figure. The concentration values measured in both sediment and larvae showed fluctuations due to differences in water characteristics from each observation station, both physical and chemical properties **(Dewi *et al.*, 2014; Takarina, 2014; Edward, 2020; Yang *et al.*, 2020)** as well as the ability of Hydropsychidae larvae to accumulate each type of metal **(Tszydel *et al.*, 2016)**.

Several previous studies have shown a proportional trend between the concentrations of heavy metals in water or sediments and their concentrations in the bodies of invertebrate larvae. However, in this study, there was a difference between the heavy metal concentration values in the sediment and the concentration in the larval tissue. Different gradient patterns occur in metal concentrations in the sediment and the bodies of the larvae, as seen in Figs. (2, 3). This gradient pattern may only indicate the level of bioavailability, not the concentration of metals in the sediment. Although the concentration of Pb in sediments is much higher than that of Hg and Cd, the concentration of Pb can only accumulate about 1% in the body. On the other hand, the concentration of Hg and Cd in sediment accumulates around 2× in the body of biota. This is supported by the results of BCF calculations, which illustrate the ability of larvae of *Cheumatopsyche* sp. to mobilize Pb (BCF<1) from sediments into low tissue.

Heavy metals can accumulate in the tissues of aquatic organisms. Animals absorb heavy metals through food and accumulate in tissues **(Castro *et al.*, 2009; Arsad *et al.*, 2021)**. Continuous exposure, even at low concentrations, limits self-purification capabilities and reduces heavy metal concentrations in tissues **(Cain *et al.*, 2006; Chiba *et al.*, 2011)**. Hydropsychidae are generally sedentary filter collectors, so the high accumulation of heavy metals in larval tissue is likely caused by ingesting contaminated materials suspended in water or deposited to the bottom of the river bed. The presence of heavy metals in the bodies of Hydropsychidae larvae can also be affected by artificial fertilizer waste or plant protective chemicals, which usually contain Cd, Zn, Cu, Ni, and

Pb **(Sudarso *et al.*, 2013; De Girolamo *et al.*, 2017)**. The presence of lead (Pb) and cadmium (Cd) in the bodies of *Cheumatopsyche* sp. larvae appears to be attributed, at least in part, to agricultural activities in the vicinity of the Bone River. In addition, the long life cycle, sedentary behavior, and omnivorous causes these larvae to accumulate heavy metals from water, bottom sediments, and the surrounding biocestone **(Cain & Luoma, 1998)**.

One of the effects of metal pollution on Trichoptera is the occurrence of morphological abnormalities in these animals. High pollution levels in freshwater ecosystems have been known to increase the incidence of morphological abnormalities in freshwater animals. Morphological abnormalities of aquatic insects have long been used in studies related to the effects of toxic pollutants on aquatic ecosystems **(Yoga, 2014; Sudarso & Yoga, 2015; Tszydel *et al.*, 2021)**. Cell abnormalities are triggered by stressors originating from the environment, normal cells will experience damage or death when they cannot adapt to the existence of these stressors. The identified cell damage in several aquatic invertebrates contaminated with heavy metals was necrosis, atrophy, and vacuolization **(Amaral *et al.* 2006; Odendaal & Reinecke, 2007; Sudarso & Yoga, 2015; Widiastuti, 2019)**. Figs. (4, 5) show changes in the shape and size of the lumen diameter of the larvae as an indication of vacuolization and cell damage due to heavy metal contamination.

The digestive tract of aquatic insect larvae generally has a tube-like shape that extends to the anus and is surrounded by special gland cells called chloragogen cells **(Morgan *et al.*, 2002)**. River organic materials and heavy metals can accumulate in the bodies of Trichoptera larvae **(Sudarso *et al.*, 2013)**, whereas in *Cheumatopsyche* sp., they can accumulate in the digestive organs causing tissue damage, as indicated by changes in the surface area of the lumen damage **(Yoga, 2014)** and the reduced number of chloragogen cells **(Widiastuti, 2019)**. Moreover, heavy metals can impact aquatic organisms' physiology **(Kadim & Arfiati, 2022)**. Changes in the surface area of the lumen in tissues are a mechanism for dealing with heavy metals in large quantities and how to dispose of them through cell decay **(Morgan *et al.*, 2002)**. The results of observations using the SEM microscope in this study confirmed that heavy metals were absorbed in the body or tissues of the larvae of *Cheumatopsyche* sp. Fig. (6) shows that part of the tissue in the epithelial layer turns white, which is possible due to the presence of heavy metals that are also absorbed.

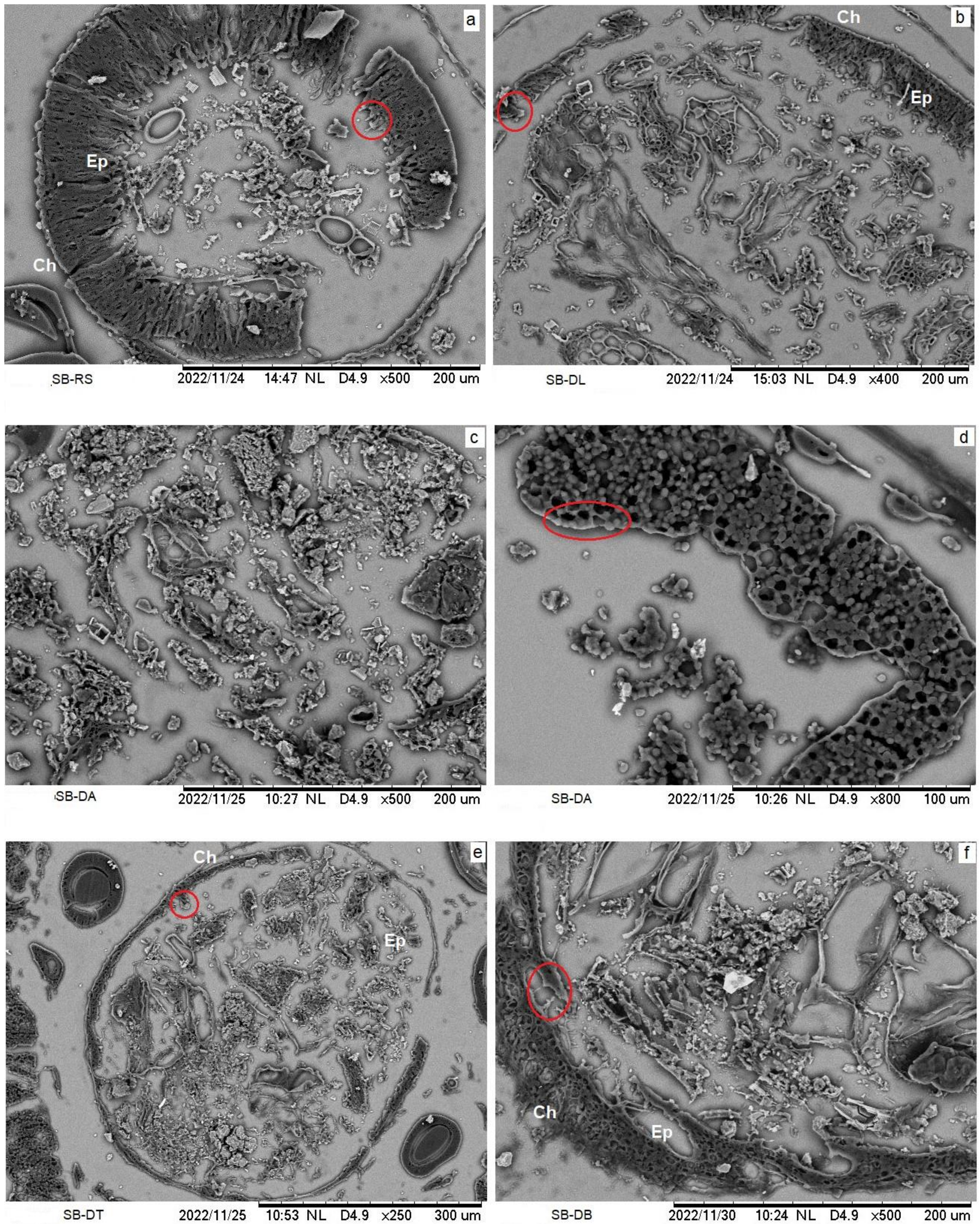

Fig. 6. SEM photo of the lumen of the larvae of *Cheumathopsyche* sp., the appearance of epithelial cells turning white due to heavy metals (red circle). a-f indicates sample images at each sampling station. Ep = Epithelial Cells, Ch = chloragogen cells

The intensity of the white color seems to increase with increasing heavy metal content in the tissue. This can be seen through a comparison of the appearance of the lumen section of the larval sample from the reference site (Fig. 6a) with the lowest concentration of heavy metals in the sediment and tissue among all stations to the appearance of the lumen in the larval sample from the SB-DT area (Fig. 6e). The appearance of the lumen of the larvae from the reference site has an epithelial layer that is still thick and compact, in contrast to the lumen of the larvae from the SB-DT area, which shows an enlarged cavity so that the epithelial layer thins and separates. According to **Morgan *et al.* (2002)**, heavy metals that enter the body of organisms can be absorbed in the tissues due to the body's metabolic activity or are carried along by the bloodstream and then distributed throughout the tissues. The response of aquatic insects to heavy metals can be more complex than looking at their presence or absence in a location or the concentration of heavy metals within the biota's body. Often, the effects are not acute but chronic and subtler **(Tszydel *et al.,* 2016)**. Tissue damage due to the accumulation of heavy metals in the bodies of aquatic insects can lead to metabolic disturbances that potentially reduce the biota's ability to maintain its survival **(Kadim *et al.,* 2013; Sudarso *et al.,* 2013)**. Ultimately, this condition affects the population and biomass of the biota.

It is generally noted that heavy metal concentrations in the tissues of Hydropsychidae larvae are associated with sediment contamination in locations where the source of pollution is agriculture **(Girgin *et al.,* 2010)** or urbanization industry **(Poteat *et al.,* 2013)**. Hydropsychidae larvae are a group of organisms tolerant to metals and can accumulate heavy metals in body tissues even if the environmental elements are in low concentrations **(Tszydel *et al.,* 2015)**. While there typically exists a positive correlation between the presence of heavy metals in water or sediment and their accumulation in the tissues of organisms, it is important to note that this may not hold true for all elements that were identified. Multiple elements were detected in the larvae of *Cheumatopsyche* sp., and their accumulation patterns can vary such as C, O, Na, and S (Table 3), which are essential organic elements needed by organisms in the body's metabolic processes. Silicon (Si) is a non-organic element that is also utilized by organisms. Zinc is an important mineral the body uses to help the growth process. In this study, Pt, Na, Zr, Si, and S were also detected. None of these elements were detected in previous studies in the Bone River, making it difficult to assess whether metals were present in the water. **Tszdel *et al.* (2021)** in his research also found elements that had not previously been detected in the anal papillae of *Hydropsyche* sp. larvae; this was made possible by contributions to food absorption or originating from elements at the sample preparation stage or trace amounts previously not exceeding the detection threshold.

The occurrence of specific heavy metals within larvae's bodies can be attributed to the degree of element assimilation and the effectiveness of elimination when the concentration reaches toxic levels **(Cain *et al.,* 2006)**, bottom sediments **(Stephansen *et al.,* 2016)** and duration of exposure to pollution **(Tszydel *et al.,* 2016)**. Aquatic invertebrates have organs with the ability to 'record' water pollution **(Sudaryanti *et al.,* 2001)**. These organs include midgut epithelium, malpighi tubules, or adipose tissue

(Johnson *et al.*, 1993), and external organs such as anal papillae **(Tszydel *et al.*, 2021)**. The appearance of visible changes in these organs may be the first sign for further environmental investigation.

CONCLUSION

In this study, the larvae of *Cheumatopsyche* sp. including low-category bio-accumulators tend to accumulate Hg as well as Pb and Cd. Through observations using the SEM-EDX microscope, we were able to confirm that there are morphological alterations in the bodies and tissues of *Cheumatopsyche* sp. larvae. These changes serve as conclusive evidence of the uptake of heavy metals in the larvae's bodies. Consequently, *Cheumatopsyche* sp. larvae can effectively serve as bio-indicators for the presence of heavy metals in river waters.

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