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First record of heavy metal concentrations in the natural habitat of silkworms, Riau Province, Indonesia

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ABSTRACT

Silkworms (*Tubifex* spp.) obtained from contaminated habitats are used as live food by fish culture experts in Riau Province. The main supply of these worms comes from the Sail River in the middle of Pekanbaru City and has received a lot of organic and inorganic waste such as heavy metals from domestic activities and the city's economy. Heavy metal content in this worm habitat is almost limited to be investigated to answer the possibility of fish mortality by fish farmers in the future. This study aims to investigate the quality conditions, presence of heavy metals in water, sediment, and bioaccumulation in these silkworms from three worm collection centers. Research data revealed that the presence of heavy metals in water with the largest to the smallest concentration order is Zn> Pb> Fe> Cr> Cu> Cd and the highest concentration in Zn ranges from 0.6364 ± 0.14 to 1.6738 ± 0.16 mgl-1 and the lowest at Cd between 0.007 ± 0.003 to 0.0178 ± 0.005 mgl-1. This metal is also found in sediments with the largest to the smallest concentration of Zn> Fe> Cr> Cu> Pb> Cd with Zn being the highest ranging from 63.8896 ± 1.23 to 121.1536 ± 1.31 mg kg-1 and the lowest is Cd ranging 0.4642 ± 0.14 to 0.9618 ± 0.11 mg kg-1. The concentration of heavy metals in worms is higher, especially essential heavy metals than in water and sediment with the order Zn> Fe> Cu> Cr> Pb> Cd and their respective concentration ranges are Zn 84.5556 ± 1.50 to 92.8354 ± 1.70 mg kg-1, Fe 41.0664 ± 2.78 to 61.3076 ± 2.42 mg kg-1, Cu 6.8536 ± 0.58 to 15.7204 ± 0.46 mg kg-1, Cr 4.4846 ± 0.39 to 8.9826 ± 0.41 mg kg -1, Cd 0.4148 ± 0.12 to 1.9784 ± 0.13 mg kg-1 and Cd 0.4148 ± 0.12 to 1.9784 ± 0.13 mg kg-1. The BAF value shows that the highest accumulation of heavy metals into the body of silkworms comes from water as compared to sediment at all locations. This study shows that the worms and the natural habitat they collect are contaminated with heavy metals, but are still suitable as live food in fish hatcheries.

Keywords : ***T****ubifex, Bioaccumulation, Sediment, Water quality, Sail River*

Introduction

Indonesia's fishery production in 2015-2019 has increased from 22.3 million tonnes to 23.86 million tonnes and of this total, freshwater aquaculture contributed 5.88 million tonnes (Ministry of Marine Affairs and Fisheries of the Republic of Indonesia, 2019). The increase in national fishery production cannot be separated from the contribution of fishery production in each province, such as Riau Province. This province contributes to national fishery production of 348,704.3 tons (1.52%) with an export value of US $ 4,310,900 originating from aquaculture production of 61.37% (Department of Maritime Affairs and Fisheries Riau Province, 2018) (Liana *et al.*, 2014).

This silkworm (*Tubifex* spp) lives in abundance on the bottom of slow-flowing waters with a physical appearance such as an attractive stretch of waving red carpet. Generally, all fish hatchery experts are still loyal to using these worms as a cheap and highly nutritious alternative natural feed to deal with factory feeds that tend to be high. These worms have a protein content of 52.49-57% and fat 13-13.3% (Bintaryanto and Taufikurohmah, 2013; Subandiyah and Satyani, 2017), are slow-moving, small in size, and easy to digest (Cahyono *et al*., 2015). Also, it has a short growing period in various habitats and tolerates a wide spectrum of environmental variables (Birtwell and Arthur 1980; Brinkhurst and Kennedy 1965; Kaster, 1980), and has high fecundity (92 - 340 eggs) and the capacity to reproduce. in a temperature range of 0.5 – 30 °C (Poddubnaya, 1980).

The production center for silkworms from natural habitats in Riau Province is the Sail River, Pekanbaru City. This river is a tributary of the Siak River with a watershed area of 135.8 km2 and crosses 4 sub-districts (Tenayan Raya, Bukit Raya, Sail, and Lima Puluh), but 3 of them represent the locations for collecting these worms. For more than 35 years, the main function of this river is no more than a channel for draining and collecting wastewater from most of the residential areas and economic activities of Pekanbaru City as well as rainfall-runoff. Settlements of people with various economic activities are directly adjacent to the banks of this river so that the width of the river is getting narrower and added to the occurrence of erosion and silting, thereby reducing the water holding capacity which implies flooding, especially in the downstream segment.

About 3.5 - 4 km from the downstream of this river, there are 50 worm collecting fishermen with the production of clean red worms reaching around 1,000-15,000 cans of sweetened condensed milk per day. The selling price of these worms ranges from IDR 12,000-15,000/can or IDR. 60,000-75,0000 per kg. The turnover of money from this worm business is estimated at IDR 15-22.5 million/day (IDR 3.6-5.4 billion/year) with the main marketing area in Kampar Regency, apart from Pekanbaru, Pelalawan, Siak, Dumai, Bengkalis, and Indragiri Hulu. It is feared that the provision of these worms as natural food will affect the health conditions of fish seeds. So far there have been complaints personally from fish hatchery experts in Riau but this is not an official report. From various scientific investigations, it has been shown that these soft benthic animals live in basic sediments to become bioindicators of organic pollution and accumulate heavy metals or other harmful pollutants and act as carriers for many parasites/diseases for fish.

Weak health and mortality of fish given the contaminated silkworms can be attributed to the presence of heavy metal concentrations from their natural habitat in the Sail River. This river is known to have long been exposed to heavy metals (Pb, Cd, Cr, Zn, Cu, and Fe) with high concentrations and organic pollution with BOD values of 10.3-67.2 mgl-1 and COD of 43.6-369.5 mgl-1(Pekanbaru City Environment Agency, 2007 and 2017). Research by Singh *et al*. (2007) confirms that silkworms taken from the coastal districts of Mumbai and Thane are not suitable for feeding fish or crustaceans in hatcheries because Pb, Cd, Cu, Fe, and Zn are found in water, sediment, and their bioaccumulation in the body of these worms. very high.

Benthos play an important role in water food webs and are known to be very sensitive to heavy metals that accumulate in the body tissues of organisms (Singh *et al*., 2007) and can affect the immune system of invertebrates (Burch *et al*.,1999). Heavy metals, such as Hg, Cd, Cu, Pb, and Zn are the most important pollutants affecting the aquatic environment and fish. They are very dangerous for fish health. These metals can effectively affect the vital operations and reproduction of fish; weaken the immune system, and causes pathological changes (Authman *et al*., 2015).

The natural habitat of silkworms in most parts of the country has been almost limitedly investigated and associated heavy metals are being paid more attention in waters and fish and terrestrial organisms. The first recording of this study aims to characterize the physico-chemical properties of water, the presence of heavy metals in water and sediments and their bioaccumulation in silkworms from natural habitats in the Sail River which have been used for decades but are still very limited.Therefore, this study is important because it provides basic data to answer when complaints or reports related to the impact on fish health are peaked by fish culture experts in this country.

Materials and Method

Description of the studysites

The research station in Figure 1 consists of 3 locations which are the collection locations for silkworms by fishermen on the Sail River (a tributary of the Siak River) with a sequence of locations 1, 2, and

3 at the following coordinate positions, namely: 0030'48 "LU 101028'12 East Longitude; 0031'32 "LU 101027'58" BT; 0032'29 "LU 101028'5" BT. At these locations measurement and sampling of various parameters such as temperature, current velocity, brightness and depth, pH, DO, CO2, nitrate, phosphate, free ammonia, and heavy metals (Pb, Zn, Cd, Cu, Cr, Fe) were carried out from the water, sediment, and Tubifex worm body tissue.

Sampling

Water, sediment, and Tubifex worm samples were collected at a distance of 2 meters from three subsampling points at each sampling location at 09.00 WIB in October-December 2017 and January-March 2018. Air samples were collected using BOD bottles (250 ml) that had been acidified, the sediment (one kg wet weight) was collected by hand and the worms were under slow running water and the collected samples were frozen at 4°C before analysis. Water pH using a pH meter (Hanna HI98107 water pH, based on pocket microprocessor pH tester), N-nitrate, free ammonia, and phosphate were analyzed using a spectrophotometer. DO and CO_2 were analyzed following standard analysis methods (Trussell 1989). The temperature was measured with an alcohol thermometer (rod), corrected Secchi disk, and current velocity with manual flow drogue and depth using a scaled stick.

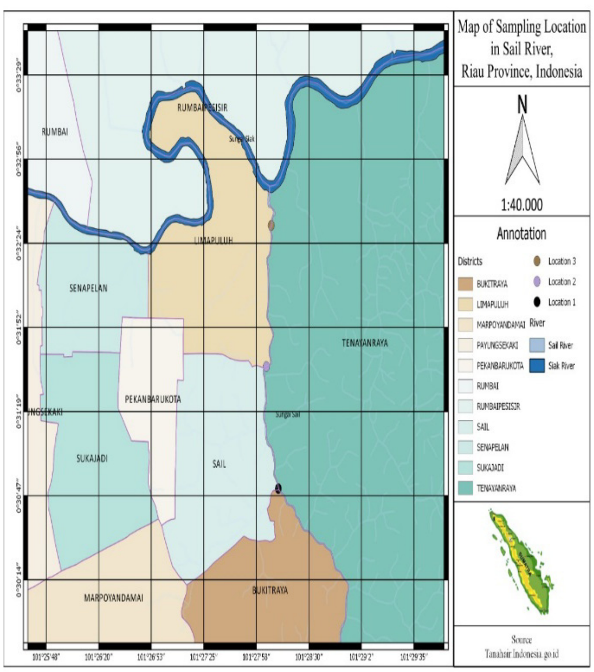

Fig.1. Location of Sail River, Pekanbaru City, Indonesia

Tubifex worm digestion refers to Singh *et al*. (2007) that is, about 200 g of all tubifex worms from each collection site before analysis, sun-dried, and sediment before constant weight was achieved. One gram of analyte was weighed using an analytical balance (1 g ± 0.0001 g) and mixed with 10 ml of HNO_3 and stored overnight. Then it was mixed with 10 ml of a 4: 1 ratio of HNO_3 solution and perchloric acid, then heated on a hotplate until the sample was reduced to a white residue. After cooling, the residue was dissolved in triple distilled water and the volume was made up to 100 ml in a volumetric flask for further analysis of heavy metals. For the analysis of heavy metal concentrations in water, the sample was filtered using Whatman filter paper and 50 ml of the sample was used for aspiration. Atomic absorption spectrophotometer (Shimadzu, AA-7000 type flame) was used to analyze the concentration of heavy metals in water, sediment, and body tissue of tubifex worms with the characteristic wavelengths of Cu (324.8 nm), Zn (213.9 nm), Fe (248.3 nm), Cd (228.8 nm), Cr (357.9 nm) and Pb (217.0 nm).

Statistical analysis.

Water quality data (physico-chemical), including heavy metals (Pb, Cd, Cr, Cu, Fe, and Zn) were analyzed by comparing with air quality standards (Government Regulation of the Republic of Indonesia, 2021) and sediment (Anzecc, 2000), and an analysis of the accumulative level of heavy metals in the worm's body was carried out based on the BAF (bioaccumulation factor) value.

Results

The average density of red worms at locations 1, 2, and 3 was 376405 ind per m^2, 352211 ind per m^2, and 384305 ind per m^2, respectively. These worms live with the following water quality characteristics: water temperature ranges from 28.38±0.29 to 29.78±0.37 °C, brightness 8.26±1.94 to 12±1.22 cm, current velocity 0.0489±0.03 to 0.251±0.05 m/sec, depth 0.9778±0.43 to 1.7111±0.42 m, water pH 5.7889±0.34 to 6.0222±0.17, DO 1.9489±0.35 to 2.5956±0.33 mgl^{-1}, CO_2 8.0489±3.38 to 14.0833±3.42 mgl^{-1}, nitrate 0.0989±0.04 to 0.1337±0.04 mgl^{-1}, free ammonia 0.1699±0.07 to 0.3115±0.06 mgl^{-1}, phosphate 0.3866±0.16 to 0.4572±0.20 mgl^{-1} and TOM 4.4151±0.18 to 6.907±0.17 % with categories of sediment fractions from sandy mud to muddy sand, respectively are presented in Table 1.

Heavy metals in water and sediment.

The data of heavy metal concentrations in water are Zn>Pb>Fe>Cr>Cu>Cd (Table 2). Zinc (Zn) is in the range 0.6364±0.14 to 1.6738±0.16 mgl^{-1}, lead (Pb) 0.22298±0.08 to 0.7908±0.08 mgl^{-1}, iron (Fe) 0.4158±0.06 up to 0.8974±0.06 mgl^{-1}, chromium (Cr) 0.115±0.04 to 0.1838±0.05 mgl^{-1}, copper (Cu) 0.0053±0.001 to 0.0900±0.006 mgl^{-1} and cadmium (Cd) 0.007±0.003 to 0.0178±0.005 mgl^{-1}. The content of all heavy metals in water was observed to increase from location 1 to location 3 and was above the water quality standards for Class II and III, namely: Zn 0.05 mgl^{-1}; Pb 0.03 mgl^{-1}; Fe 0.3 mgl^{-1}, Cr 0.05 mgl^{-1}, Cu 0.02 mgl^{-1} and Cd 0.01 mgl^{-1}(Government Regulation of the Republic of Indonesia, 2021).

Most of the heavy metal concentrations in the sediment have the same sequence pattern as water, except for Pb and Fe in the following order: Zn>Fe>Cr>Cu>Pb>Cd (Table 3). The concentration of Zn was highest with a range between 63.8896±1.23 to 121.1536±1.31 mg kg^{-1} followed by Fe 25.5648±1.30 to 39.8510±1.47 mg kg^{-1}, Cr 15.1402±1.09 to 22.1646±1.40 mg kg^{-1}, Cu 7.4254±0.76 to 18.7062±0.82 mg kg^{-1}, Pb 1.3326±0.32 to 3.4342±0.38 mg kg^{-1}, and the lowest is Cd 0.4642±0.14 to 0.9618±0.11 mg kg^{-1}. Heavy metals in sediment also increased from location 1 to location 3 as well as in water, but the concentration was still below the heavy metal standard in sediments (Anzecc, 2000).

The concentrations of various heavy metals in the Tubifex worm body from each station (Table 4) in order of heavy metal concentrations from the largest to the smallest were Zn>Fe>Cu>Cr>Pb>Cd and essential heavy metals were higher than non-essential. Zn concentrations ranged from 84.5556±1.50 to

Table 1. The environmental parameters (mean ± standard deviation) at Natural Habitat Locations for Silkwroms Collection

Parameters	Location 1	Location 2	Location 3
Temperature (°C)	28.38 ± 0.29	28.59 ± 0.36	28.69 ± 0.37
Water brightness (cm)	8.26 ± 1.94	10 ± 2.52	12 ± 1.22
Flow Velocity (m/sec)	0.1677 ± 0.05	0.1656 ± 0.06	0.0489 ± 0.03
Depth (m)	1.7111 ± 0.42	1.3889 ± 0.54	0.9778 ± 0.43
pH	5.7889 ± 0.34	6.0222 ± 0.17	5.7444 ± 0.40
DO (mgl^{-1})	1.9489 ± 0.35	2.3633 ± 0.26	2.5956 ± 0.33
CO_2 (mgl^{-1})	8.0489 ± 3.38	11.4156 ± 3.35	14.0833 ± 3.42
Nitrate (mgl^{-1})	0.0989 ± 0.04	0.1284 ± 0.06	0,1523 ± 0.04
Ammonia (mgl^{-1})	0.1699 ± 0.07	0.1845 ± 0.07	0.3115 ± 0.06
Phosphate (mgl^{-1})	0.4572 ± 0.20	0.3866 ± 0.16	0,4841 ± 0.18
Total Organic Matter (%)	6.907 ± 0.17	6.5999 ± 0.13	4.4151 ± 0.18
Sediment Fraction			
- Gravel	3.823 ± 0.11	7.637 ± 0.08	4.023 ± 0.10
- Sand	39.043 ± 0.46	46.973 ± 0.39	38.103 ± 0.43
- Mud	57.133 ± 0.46	45.39 ± 0.44	57.874 ± 0.41
- Category	Muddy sand	Sandy mud	Muddy sand

Table 2. Heavy Metal Concentrations (mean±standard deviation) in Water at Natural Habitat Locations for Silkworms Collection.

Heavy Metal Concentrations (mgl^{-1})	Location 1	Location 2	Location 3
Pb	0.2398 ± 0.08	0.4934 ± 0.07	0.7908 ± 0.08
Zn	0.6364 ± 0.14	1.5834 ± 0.14	1.6738 ± 0.16
Cd	0.007 ± 0.003	0.0096 ± 0.002	0.0178 ± 0.005
Cu	0.0053 ± 0.001	0.0420 ± 0.006	0.0900 ± 0.006
Cr	0.1150 ± 0.04	0.1476 ± 0.04	0.1838 ± 0.05
Fe	0.4158 ± 0.06	0.7528 ± 0.05	0.8974 ± 0.06

92.8354±1.70 mg kg^{-1} followed by Fe 41.0664±2.78 to 61.3076±2.42 mg kg^{-1}, Cu 6.8536±0.58 to 15.7204±0.46 mg kg^{-1}, Cr 4.4846±0.39 to 8.9826±0.41 mg kg^{-1}, Cd 0.4148±0.12 to 1.9784±0.13 mg kg^{-1} and Cd 0.4148±0.12 to 1.9784±0.13 mg kg^{-1}. The concentration of heavy metals in the worm's body also increases based on the order of location such as the concentration of heavy metals in water and sediment.

Accumulation of heavy metals into the body of these silkworms through respiration, food, and skin from water and sediment sources is indicated by the BAF (bioaccumulation factor) value of each location (Table 5). Based on the BAF value (Table 5), it was found that the highest accumulation of heavy metals into the body of silkworms came from the water medium at all observed locations compared to sediments with the sequence pattern of heavy metals based on the location being Cu>Zn>Fe>Cd>Cr> Pb (location 1), Cu>Cd>Fe>Zn>Cr>Pb (location 2) and Cd>Cu>Fe>Zn>Cr>Pb (location 3), where the sequence pattern is different from the sequence pattern of heavy metal concentrations in water, sediment, and this worm's body. In locations 2 and 3, it was found that silkworms accumulated more non-essential toxic metals such as Cd, reaching a range of 72 - 118 times their concentration in water medium compared to location 1, which mostly accumulated essential heavy metals, such as Cu, Zn, and Fe with their accumulation levels. respectively 1382, 137, and 100 times their concentration in water. Meanwhile, the accumulation rate of heavy metals Cr and Pb at the three locations was the lowest at the three locations.

Discussion

Physico- chemical characteristics of water

In this research, silkworms can live and thrive at acidic pH, dissolved O_2 concentration, and low water transparency with a slow water flow rate and CO_2, nitrate, phosphate, and free ammonia which are quite high. Among the parameters such as pH, O_2, CO_2, nitrate, and phosphate as well as free ammonia can be an indication that this river has received a continuous organic waste discharge and is influenced by the input of peat river water during high tide from the Siak River, especially at location 3 as the largest location.

The physical and chemical conditions of water in this finding are lower than the habitat conditions for worms in Mumbai, India (Singh *et al*., 2007) which are contaminated with sewage (Kailasam *et al*., 2002). The O_2 concentration of 1.9 mg / l from this study was higher than the minimum level of 1.7 mg

Table 3. Heavy Metal Concentrations (mean ± standard deviation) in Sediment at Natural Habitat Locations for Silkworms Collection.

Heavy Metal Content ($mgkg^{-1}$)	Location 1	Location 2	Location 3
Pb	1.3326 ± 0.32	2.0892 ± 0.55	3.4342 ± 0.38
Zn	63.8896 ± 1.23	63.9744 ± 1.14	121.1536 ± 1.31
Cd	0.4642 ± 0.14	0.8280 ± 0.11	0.9618 ± 0.11
Cu	7.4254 ± 0.76	10.4712 ± 0.86	18.7062 ± 0.82
Cr	15.1402 ± 1.09	21.6430 ± 1.71	22.1646 ± 1.40
Fe	25.5648 ± 1.30	34.1758 ± 1.52	39.8510 ± 1.47

Table 4. Heavy Metal Concentrations (mean±standard deviation) in Silkworms Body Tissue at Natural Habitat Locations.

Heavy Metal Content ($mgkg^{-1}$)	Location 1	Location 2	Location 3
Pb	4.2268 ± 0.87	5.4392 ± 0.85	7.9184 ± 0.89
Zn	84.5556 ± 1.50	87.7502 ± 1.68	92.8354 ± 1.70
Cd	0.4148 ± 0.12	0.7300 ± 0.13	1.9784 ± 0.13
Cu	6.8536 ± 0.58	9.3048 ± 0.66	10.3048 ± 0.46
Cr	4.4846 ± 0.39	5.8892 ± 0.44	8.9826 ± 0.41
Fe	41.0664 ± 2.78	49.8796 ± 2.87	61.3076 ± 2.42

Table 5. BAF Heavy Metal Value by Silkworms

Location	Heavy Metals	Bioaccumulation Factor (BAF)	
		Organisms - Water	Organisms - Sediment
1	Pb	18.166 ± 2.529	3.204 ± 0.369
	Zn	137.189 ± 25.337	1.324 ± 0.033
	Cd	62.546 ± 14.529	0.908 ± 0.115
	Cu	1381.744 ± 42.535	0.925 ± 0.035
	Cr	41.965 ± 12.355	0.298 ± 0.039
	Fe	99.88 ± 9.675	1.609 ± 0.121
2	Pb	11.068 ± 1.422	3.204 ± 0.369
	Zn	55.910 ± 5.924	1.324 ± 0.0326
	Cd	76.968 ± 11.742	0.908 ± 0.035
	Cu	224.091 ± 25.553	0.925 ± 0.035
	Cr	42.335 ± 11.038	0.298 ± 0.039
	Fe	66.387 ± 3.742	1.609 ± 0.121
3	Pb	10.013 ± 0.419	7.918 ± 0.895
	Zn	55.802 ± 4.357	92.835 ± 1.695
	Cd	118.239 ± 37.335	1.978 ± 0.131
	Cu	117.337 ± 7.360	10.531 ± 0.462
	Cr	52.869 -± 18.209	8.983 ± 0.412
	Fe	68.430 ± 2.778	61.308 ± 2.418

/ l required by tubifex worms (Marian *et al*., 1989). Tubifex can survive at low O2 concentrations, and can even survive without oxygen for a long time, and has aerotorxic characteristics (Pennak, 1989). From various articles, it is known that *Tubifex tubifex* is one of the oligochaete species that are most tolerant of high or low temperature, salinity, and pH can survive anaerobic conditions (Smith, 2001), making it a perfect indicator species of organic pollutants such as urban sewage and runoff and water quality indicators.

At this location, tubifex worms inhabit a substrate of sandy mud (sites 1 and 3) and muddy sand (sites 2) with a high content of total organic matter. This category of sediment plays a major role in the distribution and abundance of tubifex at this location. Tubifex is one of the studied Oligocaheta species and shows a preference for substrates with high clay and silt content and also on sandy substrates with less than 10% clay and silt (Sauter and Güde, 1996). This corresponds to the particle size of the resulting fecal pellets, meaning these species actively select the sludge-clay fraction and avoid sand particles Rodriguez *et al*., 2001). The habitat of this worm location is consistent with that revealed from previous research that *T. tubifex* prefers nutrient-rich sediments (ie high organic C and total N) regardless of particle size (Wachs, 1967). It is even claimed that these species prefer coarse-grained nutrient-poor sediments (Zahner, 1967), where the correlation between their abundance and the quantity and quality of total organic matter in sediments should be investigated further at this location.

Heavy Metals in Water and Sediment

The concentrations of heavy metals observed in the water are higher than their natural concentrations, which confirms that the source comes from anthropogenic activities on land. Like a river in the middle of an urban area that has received waste disposal from settlements, workshops, agriculture, restaurants, metal coatings, hospitals, sand mining, etc. from the city directly or through hundreds of drainages that empties into this river and is surrounded by highways. as a major contributor of heavy metals in silkworm habitat locations. Concentrations of Pb, Cd, Cu, and Fe in the natural habitat of these worms are lower than the results of monitoring by the Environmental Agency of Pekanbaru City (2007, 2009, 2013, 2014, 2015, 2017) with Pb values of 0.009 – 2.526 mg l-1 , Cd 0.002 – 0.02 mg l-1, Cr 0.002 – 0.103 mg l-1, Zn 0.014 – 0.874 mg l-1, Cu 0.002 – 0.218 mg l-1 and Fe 0.192 – 1.215 mg l-1, except for Cr and Zn. Among heavy metals such as Pb and Zn, it is higher than Pb (0.211 – 0.7870 mg l-1) and Zn (0.1320 – 0.2775 mg l-1) in the Siak River (Budijono *et al*., 2016). In the fish *Notopterus notopterus* measuring 22.9 – 28.0 cm that inhabits this river has accumulated high Cd (2.95 mg/kg) in muscle, followed by Cr (95.62 mg/kg) in liver and Pb (35 mg/kg) /kg) on the gills. Meanwhile, in the same fish with a size of 12.7 – 17.8 cm, the highest accumulation of heavy metals was found for Zn (88.47 mg/kg) and Fe (2.35 mg/kg) in the gills and Cu (15.83 mg/kg) on bone(Budijono *et al*., 2020).The presence of heavy metals in this river, especially Pb, Cd and Zn is much higher than in the water and six types of fish in the Koto Panjang reservoir (Budijono and Hasbi, 2021).

Zinc and its compounds are widely used in commerce and medicine with common sources being galvanized iron, zinc chloride in plumbing, and paints containing zinc. Pb can occur naturally, but the anthropogenic source comes from Pb-based paints and leaded gasoline (Mager *et al*., 2011; Monteiro, 2011) or corrosion of old lead pipes, pesticides, through precipitation, falling lead dust, road runoff, and municipal wastewater(Sepe *et al*., 2003; Sorensen, 1991). Fe is prevalent from industrial and mining wastes. Cr from metal plating, metal finishing, mining, dyeing and printing industry, photography, and pharmaceuticals (Abbas and Ali, 2007; Arunkumar *et al*., 2000). Cu from the use of fungicides, algaecides, molluscicides, insecticides, and extensive waste disposal (Michael, 1984). Cd is also derived from agricultural chemicals, pesticides, and sewage sludge on agricultural land (ATSDR, 2003).

The concentration of heavy metals in the sediment is higher and is not always positively related to its high concentration in water. Heavy metals in sediments can be several times greater than in overlying waters directly threatening detrital benthic organisms and food and maybe a source of long-term contamination higher up the food chain (Boyd and Tucker, 1998). This is related to the travel and transfer of heavy metal ions from water into the sediments that occur. Heavy metals in the water column through various processes can settle or accumulate high in the sediment. Finally, the sediment in the aquatic environment serves as a pond that can hold a metal or release metals into the water column by various remobilization processes (Marchand *et al*., 2006), so that surface sediment is a layer that controls metal exchange between sediment and water.

Heavy metals in worms and their bioaccumulation

Silkworms can concentrate heavy metals, especially from water through the surface of the skin which is used for the respiratory process in the tail that emerges from the sediment surface compared to taking food in the sediment by the submerged head and this is indicated by the $BAF_{(organism - water)}$ > $BAF_{(organisms - sediments)}$. The bioaccumulation of Cd as a non-essential heavy metal is higher than its concentration in water and sediment so that the order pattern is tubifex worms > sediment > water, except for essential heavy metals (Cu, Zn, Cr, and Fe). The concentrations of heavy metals in both water, sediment, and tubifex worms were lower in the research, but the pattern of heavy metal sequences was similar to the heavy metal concentrations found in the natural habitat of tubifex in Mumbai, India and the highest bioaccumulation was Pb with a range of each metal. weight is as follows: Cd 2.38±0.20 – 7.21±0.82 mgkg^{-1}, Pb 14.95±0.90 – 33.49±1.26 mgkg^{-1}, Zn 60.20±1.5 – 166.20±3.2 mgkg^{-1}, Cu 29.38±1.58 – 108.90±3.5 mgkg^{-1}, and Fe 3,160±9 – 5,738±16 mgkg^{-1}(Singh *et al*., 2007). In contrast to Widiastuti *et al*. (2018) who obtained the order of Pb concentrations in air, sediment and tubifex sp are sediments > tubifex worms> air.

Bioaccumulation of essential heavy metals from this study was highest in worms at all locations because these worms lived in unfavorable environmental conditions. Essential heavy metals are known to be needed for cell and carbohydrate metabolism, a nucleic acid synthesis that occurs in many enzymes, immune systems, nerve transmission, and cell signaling (Sfakianakis *et al*., 2015; Farag *et al*., 2006; Monteiro *et al*., 2009; Celik and Oehlenschläger, 2004; Hogstrand, 2011). On the other hand, toxicity occurs either at metabolic deficiency or high concentrations (Sivaperumal *et al.*,2007). Deficiency of essential metals can cause adverse health effects, whereas high concentrations can result in negative effects that are equivalent or worse than those caused by non-essential metals (Kennedy, 2011).

The heavy metals that are absorbed into the body of the silkworms at this location are not known to harm the worms themselves, as experts think because the fact is that until now the three locations are still as worm production locations which are always taken by fishermen without stopping except during floods with intensity. high rain and this is a common use of biota in natural waters. This shows that silkworms that live in natural environments have a very high tolerance for heavy metals. According to Singh *et al*. (2007), worms that have been in this habitat for a long time have developed a certain level of immunity to these metals. The negative impact on fish fed by this worm from fish breeders or cultivators in this country has not been seen at this time because the concentration is still far below the concentration found in Tubifex in Mumbai, India so that it is still suitable as natural feed and ideally uses this natural feed from the production of worm cultivation. However, in other studies, it has been explained that worms exposed to heavy metals such as Cu, Pb, and Cd in high concentrations accumulate in the posterior end (tail) and lose this part to protect themselves from heavy metal contamination in laboratory experiments by Lucan-Bouché *et al*. (1999) and Bouché *et al*. (2000). Perhaps in this natural habitat, this reaction is carried out by worms to live in conditions contaminated with heavy metals and confirms that it can be used as a heavy metal bio-indicator.

Conclusion

This research succeeded in showing that all the silkworm collecting habitats in the Sail River were contaminated with heavy metals (Pb, Cd, Cu, Zn, Cr, and Fe) with high concentrations in water, sediment, and worm bodies and are currently considered suitable as natural food fish.

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