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HEALTH RISKS ASSESSMENT OF HEAVY METAL FROM CONSUMPTION OF *OREOCHROMIS MOSSAMBICUS* AND *OREOCHROMIS NILOTICUS* IN DENPASAR, BALI

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Abstract

Introduction: *Heavy metals in the environment can accumulate in organisms through the food chain process. Previous studies recorded heavy metal concentrations above threshold limits value in Badung river, which warrants monitoring adverse health outcomes due to consuming fish from this river. This research aimed to estimate the potential risk from heavy metals exposure, namely Pb, Cd, Cu, and Cr, due to fish consumption.* ***Methods:*** *Fish samples were limited to Oreochromis Mossambicus and Oreochromis niloticus, commonly known as tilapia. Twenty samples of composite fish muscles were collected from three fishing sites. Subsequently, the heavy metals present in the samples were quantified using an atomic absorption spectrophotometer (AAS), and health risks were assessed by calculating estimated daily intake (EDI) and target hazard quotient (THQ) for both carcinogenic and non-carcinogenic risks.* ***Results and Discussion:*** *The average concentrations of Pb in Oreochromis Mossambicus (6.35±3.21 mg/kg) and Oreochromis niloticus (6.09±3.07 mg/kg) exceed the threshold limits value for fish products, but other heavy metals remain below. The average EDI from consuming Oreochromis Mossambicus with Pb is 0.0025-0.0026 mg/kg/days; Cu is 0.0037-0.0062 mg/kg/day; Cr is 0.0001 mg/kg/day. EDI from consuming Oreochromis niloticus with Pb is 0.0015-0.0025 mg/kg/day; and 0.00 mg/kg/day for Cu and Cr. The THQ calculation for carcinogenic and non-carcinogenic health risks showed no health risk from consuming the fish from the Badung River.* ***Conclusion:*** *The study concludes that the consumption of Oreochromis Mossambicus and Oreochromis niloticus from the Badung River was generally safe from potential health risks.*

INTRODUCTION

Rivers are commonly used as disposal sites for treated and untreated wastewater from sources such as industrial facilities, agriculture, and domestic activities. The government has established regulations for wastewater to be discharged into designated bodies of water, each with its designated purposes. However, certain pollutants that enter rivers, such as heavy metals, are not biodegradable and can accumulate in the environment. While the accumulation of heavy metals generally occurs in sediments, they can also accumulate in living organisms, known as bioaccumulation.

Heavy metals have specific densities of more than 5 g/cm^3 and may impose negative impacts on the environment and organisms (1) as they enter the water systems through natural and anthropogenic processes, including soil erosion, dissolved minerals in rocks, mining activities, industrial wastewater effluents, urban wastewater, urban rainwater runoff, pesticides and other sources (1-3). Heavy metals commonly found in water bodies include arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn), all of which can potentially health risk on human and the environment (1,4).

Metals such as Cr, Cu, Zn, and Ni are essential for the functioning of biological systems but can also result in toxicities when present in excessive amounts (5-7). Heavy metals can pose health risks due to their non-carcinogenic and carcinogenic properties. Environmental Protection Agency has listed lead, cadmium, and chromium as metals that potentially cause health problems (8). For example, exposure to lead is strongly linked to category B2 health problems among animals such as neurotoxicity, developmental disorders, hypertension, hearing loss, impaired hemoglobin synthesis, reproductive disorders among men, and increased risk for cancer such as kidney tumors (8-9). The total weight of lead in the human body from environmental and occupational exposure is estimated to reach 120 mg Pb, with concentrations in the blood reaching 0.2 mg/lt (9). Another heavy metal, Cd, is categorized as having a weak potential cause of cancer in humans because no strong evidence of increased cancer risk (8). However, the liver and kidneys are sensitive to the toxic effects of Cd because these organs synthesize metallothioneins (MT) proteins which strongly bind to Cd ions during detoxification processes (10). A disease caused by exposure to Cd is the itai-itai disease resulting in bone fragility (2).

Furthermore, heavy metals such as Cr^{6+} can react with biological reducing agents to form reactive oxygen such as superoxide ions, hydrogen peroxide, and hydroxyl radicals which cause oxidative stress in body cells that can damage DNA and proteins (1). However, oral exposure to Cr^{6+} has not been proven to have carcinogenic effects (8). Similarly, Cu is categorized as having no cancer risk in humans due to a lack of evidence of its effects among animals and humans (8). The recommended reference dose for Cu intake is 0.04 mg Cu/kg/day (6-7). Regardless, as seen in Wilson's disease, the accumulation of Cu can cause liver damage and even death (6).

Heavy metals enter into organisms mainly through the food chain, where organisms at the higher trophic level will accumulate higher levels of pollutants than those at the lower trophic level, a phenomenon known as biomagnification (9). In light of this, fish being the top consumer's aquatic ecosystems, become potential sources of heavy metals for humans (11). Accumulation of heavy metals in fish vary according to the amount of heavy metals present in the surrounding environment, duration of exposure, routes of entry into the body, environmental conditions such as temperature, pH, hardness, and salt content in water, as well as intrinsic factors such the age and diet of the fish (12). Heavy metals also accumulate differently between species of fish in the same aquatic environment. Additionally, the weight of accumulated heavy metals per kilogram of fish commonly follows the order Fe > Zn > Pb > Cu > Cd > Hg (12).

International organisations such as the World Health Organization (WHO), Food and Agriculture Organization (FAO), the European Union as well as national National Agency of Drug and Food Control of Indonesia (BPOM) have set standards on the maximum concentration of heavy metals allowed in fish products (13-14). The threshold limits of heavy metal in fish products are 0.1 mg Cd/kg, 0.2 mg Pb/kg (13), and 5.5 mg Cr/kg, 20 mg Cu/kg (5). Some countries also regulate the content of essential trace elements such as Zn, Cu, Ni, and Cr because their toxic properties if present in excessive amounts (5-7).

Badung River flows from the north to the south of Denpasar, Bali, passing various sites, such as the large Badung markets, residential areas, and industrial areas of the city. As a result, the river receives a high level of pollution from these activities. Despites of pollution level on the river, some segments of this river have been used as fishing sites by surrounding residents. Previous studies have found that heavy metal concentrations such as Pb (15) and Cr (16) in the Badung River have exceeded the water quality stream standards. Abnormalities have also been noted in mullet (*Mugil cephalus*) in Badung River Estuary, which may have been caused by Cr exposure (16). Likewise, Pb and Cr^{6+} concentrations in tilapia fish in the Badung River have also been found to exceed the standards established for fish products (17). Despite ample evidence of heavy metals identified among fish in the Badung River, studies that analyze human health risks due to fish consumption remain limited.

This study aims to determine the concentration of heavy metals Pb, Cd, Cu, and Cr in *Oreochromis mossambicus* and *Oreochromis niloticus* from several segments along the Badung River Denpasar and to estimate heavy metal intake and human health risks from consuming fish from the river. Results from this study can assist the local government in reviewing current policies related to environmental pollution control and food quality control in Denpasar.

METHODS

This study employed the Environmental Health Risk Assessment (EHRA) method to estimate the human health risks from consuming fish caught in the Badung River. Twenty samples of *Oreochromis mossambicus* and *Oreochromis niloticus* were obtained from community fishing activities at three segments of Badung River, namely Tukad Korea – Kumbasari Market, Fishing Park – Pemogan, and Badung River Dam in April 2021. Samples were subsequently stored in a cool box with a temperature of ±4ºC and immediately brought to the laboratory to be weighed. Approximately ±200 g of fish muscle was collected and mashed. Samples weighing 25 grams each were placed in a porcelain dish and heated at 105ºC in an oven until a constant weight was reached to determine the water content. The dried sample was then ground with a mortar and pestle.

Heavy metal content was determined using SNI 2354.5:2011 on Determination of Heavy Metal Content in Fishery Products. The principle in testing heavy metals in fish is by releasing heavy metals from muscle tissues through dry digestion at a temperature of 105°C. Then, the sample was wet-digested by adding 1 gram of dried sample to 45 ml of distilled water, 10 ml of concentrated HNO_3 (65%) for oxidizing and metal binding, and gradually adding 5 ml of 30% H_2O_2 for fat removal. The solution was then homogenized and heated on a hot plate at 175ºC for approximately 30 minutes under a fume hood until the volume was reduced to 10 ml (7,18). After the sample had cooled, it was filtered with Whatman paper No. 42 and added with distilled water until the volume reached 25 ml. Afterward, analysis of heavy metal concentrations was performed using Atomic Absorption Spectrophotometry (AAS) with wavelengths for Cr: 357.9 nm, Cu: 324.7 nm, Cd: 228.8 nm, and Pb: 283.3 nm.

A survey among respondents who consume fish from the Badung River was conducted to gain an insight into the patterns of fish consumption in the community. Quota sampling was employed to determine the sample of respondents. The forty respondents in the study were fishing during the field survey and provided informed consent to participate. Given that fishers are commonly male, an adult female family member of each male respondent who consumes fish was recruited. The questionnaire inquired on demographic characteristics, anthropometric information, fish consumption patterns (amount and frequency of consumption), the purpose of fishing (for sale or self-consumption), and frequency of fishing to estimate the exposure from the oral route.

The environmental health risk assessment includes hazard identification, dose-response analysis, exposure analysis, and risk characteristics. Dose-response analysis was performed by first identifying the reference dose (RfD) of metal intake through oral exposure. RfD for each heavy metal was 1 μg/kg/day for Cd, 4 g/kg/day for Pb, and 40 g/kg/day for Cu (7,8). This value was used to calculate the hazard quotient (HQ) for non-carcinogenic risks, while the cancer slope factor (CSF) estimates the carcinogenic risk. Exposure analysis was performed by calculating the estimated daily intake (EDI) among male and female respondents (11), followed by calculating the hazard quotient.

The following formula calculated the EDI value, M_c was the metal concentration in fish muscle tissue (mg/kg wet weight), IR was the digestion rate calculated based on consumption patterns reported by respondents, and B_w was the average of respondents weight. EF was the frequency of exposure from the number of days of fish consumption for a year (days/year), ED was the duration of exposure from the length of exposure estimated based on the number of years that fish will consume up to a maximum age of 70 years, ED was adjusted for life expectancy in the studied community.

$$EDI = \frac{(M_C \times IR \times EF \times ED)}{B_w \times AT}$$

The non-carcinogenic risk was determined using the target hazard quotient (THQ), which was calculated using the level of fish consumption, calculated as the ratio of the EDI to the safe threshold dose (RfD) in the following equation (7,11,19).

$$THQ = \frac{(M_C \times IR \times EF \times ED)}{RfD \times B_w \times AT}$$

The following equation calculated the carcinogenic risk by (7,11), where CSF was cancer slope factor (mg/kg Bw per day). AT was the average exposure time to carcinogens, calculated by multiplying 365 days by 70 years. A THQ value > 1 and a THQc value greater than 10^{-4} indicate a health risk requiring follow-up measures (14).

$$THQc = \frac{(M_C \times IR \times EF \times ED \times CSF)}{B_w \times AT}$$

RESULTS

Heavy Metal Concentration in Fish Samples

The analysis found that the Pb content ranged from 2.76 to 12.53 mg/kg (average 6.35 ± 3.21 mg/kg) in *Oreochromis mossambicus* muscle (Table 1) and ranged from 2.32 to 11.16 mg/kg (average 6.09 ± 3.07 mg/kg meat) in *Oreochromis niloticus* (Table 2). The average Pb concentration in these two fish types exceeded the maximum limit of lead levels allowed by Regulation of National Agency of Drug and Food Control of Indonesia No. 23 of 2017 concering Maximum Limit of Heavy Metal Contamination in Processed Food (0.2 mg/kg)(13). The concentration of Cu was between 0.43 – 45.69 mg/kg muscle (average 15.32 ± 19.93 mg/kg) in *Oreochromis mossambicus* and ranged from 0.32 – 32.68 mg/kg muscle (average 8.84 ± 10.35 mg/kg muscle) in *Oreochromis niloticus* while the maximum level allowed is 20 mg/kg (5). However, there are also heavy metals that are present in lower concentrations. For example, Cr concentration ranged from being undetectable to 0.28 mg/kg muscle (average 0.32 ± 0.73 mg/kg) in *Oreochromis mossambicus* and ranging from undetectable to 0.69 mg/kg muscle (average 0.09 ± 0.19

mg/kg) in *Oreochromis niloticus,* with the maximum level allowed at 5.5 mg/kg (5). Finally, in all the samples examined, no Cadmium was detected.

Table 1. Heavy Metal Concentrations in Muscles of *Oreochromis mossambicus*

ID	Location	Concentration (mg/kg wet)			
		Pb	Cd	Cu	Cr
4	Tukad Korea	2.76	n.a	1.14	0.05
6	Fishing park	7.85	n.a	0.43	0.00
7	Fishing park	3.17	n.a	1.62	0.00
8	Fishing park	7.01	n.a	1.32	0.02
11	Dam	7.98	n.a	41.46	0.28
12	Dam	4.38	n.a	1.82	0.04
13	Dam	12.53	n.a	45.59	0.05
18	Dam	5.11	n.a	29.21	2.11
Mean		6.35*	-	15.32	0.32
SD		3.21	-	19.93	0.73
minimum		2.76	-	0.43	0.00
maximum		12.53	-	45.59	0.28
standards		0.2	0.1	20	5.5

*Note: * exceed the threshold limit, n.a. is not available*

Table 2. Heavy Metal Concentrations in Muscles of *Oreochromis niloticus*

ID	Location	Concentration (mg/kg wet)			
		Pb	Cd	Cu	Cr
1	Fishing park	8.10	n.a	1.63	0.06
2	Fishing park	7.75	n.a	0.32	0.00
3	Fishing park	11.16	n.a	4.43	0.04
5	Fishing park	3.97	n.a	2.83	0.00
9	Tukad Korea	3.56	n.a	4.12	0.08
10	Tukad Korea	2.49	n.a	0.89	0.00
14	Tukad Korea	8.57	n.a	23.31	0.04
15	Tukad Korea	3.20	n.a	32.68	0.69
16	Tukad Korea	7.17	n.a	17.92	0.14
17	Dam	4.78	n.a	3.47	0.02
19	Fishing park	10.07	n.a	4.26	0.00
20	Fishing park	2.32	n.a	10.20	0.06
Mean		6.09*	-	8.84	0.09
SD		3.07	-	10.35	0.19
Minimum		2.32	-	0.32	0.00
Maximum		11.16	-	32.68	0.69
Standards		0.2	0.1	20	5.5

*Note: * exceed the threshold limit, n.a is not available*

Fish Intake Reported by Respondents

The composition of respondents consists of 30% from Tukad Korea, 45% from the fishing park, and 25% from the Badung River Dam (Table 3). The average body weight of male respondents is 64.3±14.9 kg, and female respondents are 58.7±8.8 kg. Additionally, the average heights are 168.5±7.5 cm and 157.5±3.7 cm for males and females, respectively.

Table 4 shows the average fish size and the number of fish consumed. The average size of *Oreochromis mossambicus* consumed by the respondent's measure at 155 ± 66.55 g in weight and 16.5 ± 3.42 cm in length, and *Oreochromis niloticus* was 110 ± 46.32 g in weight and 16.5 ± 2.42 cm in length. According to respondents, reported Badung River fish intake is 71.5 g/day for *Oreochromis mossambicus* and 65.33 g/day for *Oreochromis niloticus*, with the assumption that 40% of the total fish weight is edible. Taking into account the frequency of consumption per week, the average ingestion rate (IR) of male respondents are 27.7 ± 24.6 g/day for *Oreochromis mossambicus* and 39.7 ± 29 g/day for *Oreochromis niloticus*, while IR of female respondents is 15.7 ± 12.9 g/day and 36.5 ± 31.4 g/day respectively.

Table 3. Frequency Distribution of Demographic Characteristics of Respondents

Characteristics	Male		Female	
	f=20	(%)	f=20	(%)
Location				
Tukad Korea	6	30.0	6	30.0
Fishing par	9	45.0	9	45.0
Dam	5	25.0	5	25.0
Age (mean ± SD)	31.8	9.7	31.3	10.1
Education				
Elementary education	2	10.0	4	20.0
Junior secondary education	5	25.0	5	25.0
Senior secondary education	13	65.0	11	55.0
Occupation				
Not working	1	5.0	4	20.0
Private company employee	9	45.0	9	45.0
Entreprenour	3	15.0	4	20.0
Fisherman	1	5.0	2	10.0
Other	6	30.0	1	5.0
Body weight (kg) (mean ± SD)	64.3	14.9	58.7	8.8
Height (cm) (mean ± SD)	168	7.5	158	3.7

Note: number of the respondent was 20 for each male and female group

Table 4. Average Fish Size and Consumption Patterns by Respondents

Variabels	*Oreochromis mossambicus*		*Oreochromis niloticus*	
	Mean	SD	Mean	SD
Body weight (g)	115	66.55	110.00	46.32
Lenght (cm)	16.5	3.42	16.50	2.42
Amount of fish consumed at once by male and female	1.88	0.99	1.83	1.19
Amount of muscle consumed (40% of total weight) (gr)	72.5	21.43	65.33	19.40
Consumption frequency per week				
Male	2.5	2.14	4.25	2.67
Female	1.38	0.74	3.75	2.93

Exposure Analysis

The estimated exposure was calculated as Estimated Daily Intake (EDI) and expressed in mg/kg body weight/day (Table 5) using previously calculated values. EDI values were calculated separately for the consumption of both types of fish and separately based on the sex of respondents. Components utilized in the EDI estimation including the heavy metal concentrations in fish (minimum, average, and maximum), average IR of 72.5 g/day for *Oreochromis mossambicus* and 65.33 g/day for *Oreochromis niloticus*, the average exposure frequency (EF) of 130.36 days/year among males and 71.70 days/year among females for *Oreochromis mossambicus*, EF of 221.61 days/year among males and 195.54 days/year among females for *Oreochromis niloticus,* the exposure duration from the current age of 39.88 years among male and 37.75 years among female for *Oreochromis mossambicus*, and ED of 37.08 years among male and 39.33 years among female for *Oreochromis niloticus*.

Table 5. EDI of Heavy Metal from *Oreochromis mossambicus* and *Oreochromis niloticus* from Badung River

EDI value	*Oreochromis mossambicus* (mg/kg/day)				*Oreochromis niloticus* (mg/kg/day)			
	Pb	Cd	Cu	Cr	Pb	Cd	Cu	Cr
Male								
Minimum	0.0011	n.d	0.0002	0.0000	0.0009	n.d	0.0001	0.0000
Mean	0.0026	n.d	0.0062	0.0001	0.0025	n.d	0.0036	0.0000
Maximum	0.0050*	n.d	0.0184	0.0001	0.0045*	n.d	0.0132	0.0003
Female								
Minimum	0.0007	n.d	0.0001	0.0000	0.0006	n.d	0.0001	0.0000
Mean	0.0015	n.d	0.0037	0.0001	0.0015	n.d	0.0021	0.0000
Maximum	0.0030	n.d	0.0111	0.0001	0.0027	n.d	0.0079	0.0002
Rfd	0.004	0.001	0.04	0.003	0.004	0.001	0.04	0.003

*Note: * EDI> Rfd; n.d is not detected*

The resulting EDI of lead from consuming *Oreochromis mossambicus* is 0.0026 mg/kg/day among males and 0.0015 mg/kg/day among females. Additionally, the EDI from consuming *Oreochromis niloticus* is 0.0025 mg/kg/day among males and 0.0015 mg/kg/day among females (Rfd = 0.004 mg/kg/day). The EDI of Cu from consuming *Oreochromis mossambicus* is 0.0062 mg/kg/day among males and 0.0037 mg/kg/day among females, while the EDI from consuming *Oreochromis niloticus* is 0.0036 mg/kg/day among males and 0.0021 mg/kg/day among female (Rfd = 0.04 mg/kg/day). Lastly, the EDI of Cr from consuming *Oreochromis mossambicus* is 0.0001 mg/kg/day among malesand 0.0001 mg/kg/day among female, while the EDI from consuming *Oreochromis niloticus* is 0.0000 mg/kg /day among both sexes (Rfd = 0.003 mg/kg/day).

Risk Characteristics

In this study, the non-carcinogenic THQ based on average values of all heavy metals from consuming both types of fish by male and female respondents had a value less than 1, hence categorised as having minimum health risks from exposure (Table 6). However, the non-carcinogenic THQ based on the maximum values of heavy metal concentrations of Pb in both fish species demonstrated potential health risk for male respondents (THQ>1). The hazard index (HI) of all heavy metals is 0.86 and 0.50 for males and females, respectively, from consuming *Oreochromis mossambicus* and 0.71 and 0.43 for males and females, respectively *Oreochromis niloticus*. All HI values are <1, so the risk is categorized as minimal risk. Furthermore, the calculation of carcinogenic THQ of all heavy metals studied did not suggest any health risk from consuming both types of fish by both male and female respondents (THQ < 10^{-4}), as shown in Table 7.

Table 6. Non-Carcinogenic THQ of Heavy Metal from Fish Consumption from Badung River

THQ value	*Oreochromis mossambicus*				*Oreochromis niloticus*			
	Pb	Cd	Cu	Cr	Pb	Cd	Cu	Cr
Male								
Minimum	0.2779	n.d	0.0044	n.d	0.2336	n.d	0.0032	n.d
Mean	0.6390	n.d	0.1542	0.0426	0.6134	n.d	0.0890	0.0126
Maximum	1.2614*	n.d	0.4589	0.0369	1.1230*	n.d	0.3290	0.0930
Female								
Minimum	0.1674	n.d	0.0026	n.d	0.1407	n.d	0.0020	n.d
Mean	0.3850	n.d	0.0929	0.0257	0.3696	n.d	0.0536	0.0076
Maximum	0.7600	n.d	0.2765	0.0222	0.6766	n.d	0.1982	0.0560

*Note: n.d is not detected; * THQ >1*

Table 7. Carcinogenic THQ of Heavy Metal from Fish Consumption from Badung River

THQc value	*Oreochromis mossambicus*				*Oreochromis niloticus*			
	Pb	Cd	Cu	Cr	Pb	Cd	Cu	Cr
Male								
Minimum	5.38×10^{-6}	n.d	n.a.	n.d	4.52×10^{-6}	n.d	n.a.	n.d
Mean	1.24×10^{-5}	n.d	n.a.	3.64×10^{-5}	1.12×10^{-5}	n.d	n.a.	1.08×10^{-5}
Maximum	2.44×10^{-5}	n.d	n.a.	3.15×10^{-5}	2.17×10^{-5}	n.d	n.a.	7.94×10^{-5}
Female								
Minimum	3.07×10^{-6}	n.d	n.a.	n.d	2.58×10^{-6}	n.d	n.a.	n.d
Mean	7.06×10^{-6}	n.d	n.a.	2.08×10^{-5}	6.78×10^{-6}	n.d	n.a.	6.15×10^{-6}
Maximum	1.39×10^{-5}	n.d	n.a.	1.8×10^{-5}	1.24×10^{-5}	n.d	n.a.	4.53×10^{-5}
CSF	0.0085	15	n.a	0.5	0.0085	15	n.a	0.5

Note: n.d is not detected; n.a is not available

DISCUSSION

Heavy Metal Concentration in Fish Samples

The findings in this study demonstrate that heavy metal concentrations in fish muscle were consistent with the range found in previous studies. Previous studies using samples from the same river found the concentration of Pb in fish to be higher (0.838 – 20.26 mg/kg) (17) than concentrations found in this study (2.76 – 12.53 mg/kg). Furthermore, research in Ogun River, Nigeria, found lead concentration in tilapia muscle, 7.04 – 10.5 mg/kg, within the range found in this study (20). However, some studies reported lower concentrations of lead, whereby the value found in caged fish in Changshou Reservoir, China, was 0.35 ± 0.13 mg/kg (21) and an average of 0.0144 mg/kg in fish from the Jhelum River, Kashmir Himalaya (22).

Examinations in this study were unable to detect Cd in any of the fish samples. This differs from previous findings conducted among fish from Benoa Bay, Badung River estuary, which demonstrated Cd concentration between 0.43 – 0.79 mg/kg in mullet (*Mugil cephalus*) and 0.12-0.79 mg/kg in *Siganus guttatus* (23).

The findings from Benoa Bay fish samples were consistent with the range found in Gangga River, India (0.34 – 1.32 mg/kg) (24). Meanwhile, a study in the Swat River, Pakistan, found a lower concentration of Cd, between 0.007 - 0.097 mg/kg (4). However, higher concentrations were found in tilapia meat in the Ogun River, Nigeria, ranging from 1.02 – 2.45 mg/kg (20).

In regards to Cu, the concentration found in this study was higher (0.43 – 45.69 mg/kg) than previous studies where a concentration of 0.003-0.009 mg/kg was found in fish from the Swat River, Pakistan (4), 0.2 ± 0.11 mg/kg in Chanshow Reservoir, China (21) and 0.58 ± 0.09 to 7.87 ± 2.58 mg/kg in Gangga River, India (24). Similarly, lower concentrations of Cu were also found in the study in Lake Pluszne, Poland, which ranged from 0.139 -0.393 mg/kg (25).

Meanwhile, chromium concentration in fish in this study was found to be very low (0 – 0.28 mg/kg) compared to results from Kabul River, Pakistan, which found 8.0 – 40 mg Cu/kg (3). However, chromium concentrations found in this study are similar to the results from Changshou Reservoir, China, at 0.1 ± 0.05 mg Cu/kg (21) and with the Swat River, Pakistan, which found 0.003 – 0.072 mg Cu/kg (4).

Heavy metal concentrations in fish from this study can be ranked starting from the highest to lowest concentration as follows: Pb > Cu > Cr > Cd, which corresponds to the ranking in the literature, Fe > Zn > Pb > Cu > Cd > Hg (12). Similar orders of ranking are found in Bangladesh where Ni > Pb > Cr > Cd (3) and in Swat River, Pakistan where Cd > Pb > Cr > As > Cu > Ni (4). Conversely, findings from Jinjiang River, China had a different order of heavy metal concentration where Cr > Cu > Pb > Cd (26) and also study in Chanshow Reservoir, China found with ranking Cu > Cr > Pb (21).

The location from which the fish were obtained also indicated different concentrations of heavy metals. For example, the concentration of Pb in fish samples was lower among those from upstream (Tukad Korea) than the downstream concentration (Taman Pancing and Reservoir). However, concentrations of Cu and Cr did not follow the same trend. The concentration of heavy metals in river water bodies depends on sources of pollutants, water flow rate, and particle deposition that traps dissolved inorganic compounds such as heavy metals in the sediment. Heavy metals in sediment consist of several fractions that determine the rate and route of entry into the food chain for aquatic organisms (4). The concentration of heavy metals found in fish is not only influenced by the concentration of heavy metals in the water but also influenced by the mechanism in which food or water is consumed as well as the complex mechanism of uptake and elimination of metals out of the fish body, which differs between species and organs of the fish (12). In the Indian Ganges, it was found that the concentration of heavy metals in fish organs was highest in the liver, followed by gills then meat (24), similar to those found in Lake Pluszne, Poland (25). Differences in heavy metal concentrations in fish living in rivers were also influenced by season and fishing location (22). In this study, lead concentrations in fish samples from Badung River increased downstream, according to previous studies where lead concentrations found in shellfish were higher in downstream than upstream (27), and the concentration of heavy metals in fish is higher downstream than upstream (3,28).

Exposure Analysis

The exposure analysis includes an estimate of dietary intake per day and an estimation of the average exposure per day in a year. In this study, the intake rate (IR) was determined based on a survey among male and female respondents at fishing locations. The survey results in IR values ranging from 27.7 - 39.7 g/day among males and between 15.7 - 36.5 g/day among females, as females tend to report lower levels of fish consumption than males. In some literature, the fish weight consumption among adults is 19.5 g/day (11) and 55.8 g/day (7). Differences in dietary patterns are also greatly influenced by local customs and culture, geographical location, and other socio-economic factors.

Furthermore, the heavy metal exposure from fish consumption was calculated as Estimated Daily Intake (EDI), an estimated contaminant intake in the human body through daily food consumption (11). This study found that the EDI based on the average concentrations of different heavy metals in fish samples were still below the reference dose (Rfd). Previous studies that estimated the EDI of Pb from shellfish consumption in Semarang showed a value between 0.0002 - 0.0008 mg/kg/day (27) and from fish consumption in Ganges River with an EDI of 0.0001 -0.00096 mg/kg/day (24) which is lower than that was found in this study (0.001-0.005 mg/kg/day) with an Rfd of 0.004 mg/kg/day. Meanwhile, another study in China found a very low EDI value of Pb, around 0.04 x 10^{-6} mg/kg/day (21).

This study also found a higher EDI value of Cu (0.0021 – 0.0062 mg/kg/day) from *Oreochromis mossambicus* and *Oreochromis niloticus* compared to a study in Ghana with an EDI of Cu of 0.0006 mg/kg/day from consumption of *Chrysichthys nigrodigitatus* (29). Meanwhile, a study in Poland showed a greater EDI of Cu from *Rutilus rutilus* (0.147 mg/kg/day) (25) compared to EDI of Cu in this study.

The difference in EDI with other studies can be explained by the differences in heavy metal concentrations in fish, which depends on pollution rate, and the differences in fish consumption pattern and anthropometric data from respondent surveys, both of which are influenced by cultural, socioeconomic factors. Meanwhile, in other studies, estimations were made assuming that bodyweight is around 70 kg and fish consumption is 0.019 g/person/day on average (24).

Risk Characteristics

In this study, both THQ and HI calculation, based on the average concentration of the three heavy metals, was <1. Thus, the consumption of *Oreochromis mossambicus* and *Oreochromis niloticus* from the Badung River can be categorized as safe for the community.

However, if the calculation is based on the maximum concentration of Pb, THQ, and HI values being more than 1 (one), which indicates a low potential risk to health. These findings warrant further action to improve the water quality of the Badung River to reduce public health risks associated with consuming fish from the river. Health education and promotion on health risks from consuming fish caught from the Badung River and reducing exposure to heavy metals are also important to reduce the risk. Moreover, the THQs of males tend to be higher than those of females because male respondents tend to report higher fish consumption than their female counterparts, which suggests that more intensive promotion should be targeted towards males.

The risk analysis in this study only considered heavy metal exposure from fish consumption but did not consider other sources of exposure and other portals of entry. Therefore, the health risks due to heavy metal intake are likely to be greater if it is accumulated with heavy metal exposure from other routes such as through the skin, inhalation, and from other food sources.

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CONCLUSION

The study shows that the average concentrations of Cu and Cr metals in muscles of *Oreochromis mossambicus* and *Oreochromis niloticus* caught from Badung River were still within safe limits for consumption. However, the average concentration of lead (Pb) had exceeded the standard of Regulation of National Agency of Drug and Food Control of Indonesia No. 23 of 2017 concering Maximum Limit of Heavy Metal Contamination in Processed Food (0.3 mg/kg). The health risks estimation for both non-carcinogenic and carcinogenic show that the EDI of the average concentrations of the three metals detected is still within the safe limits (THQ<1 and THQc> 10^{-4}). However, the calculation of THQ based on the maximum concentration of Pb in both fish species has shown a potential health risk of THQ>1. Actions are needed to reduce the heavy metal content in the Badung River through stringent pollution control from both industrial and domestic activities. Public education and promotion on pollution control and reducing heavy metal exposure from fish consumption are also important to mitigate long-term health risks.

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