Isolation and Analysis of Heavy Metal Content in Waste Scales from Three Species of Farmed Fish in Lake Maninjau

by Hafrijal Syandri

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Abstract. The study isolates and analyzes heavy metal content in fish scale waste from cultured *Osphronemus goramy*, *Cyprinus carpio*, and *Oreochromis niloticus* in Lake Maninjau. Fish weights (n=10) for O. goramy, C. carpio, and O. niloticus were 389.99 ± 24.96 g, 633.30 ± 87 g, and 210.5 ± 12.12 g. Isolation begins with washing scales using running water, followed by 24-hour immersion in 10% NaCl (1:10 w/w) in a refrigerator. Scales are then placed in a pressure-equipped cooker and cooked; afterward, they are oven-dried at 50°C for 3.5 hours and ground into powder. Fish scale results were analyzed using the dry weight/wet weight formula x 100. AOAC-compliant spectrometry with three replications tested heavy metals Hg, Pb, Cr, As, Ag, Cu, and Mo. Results for *O. goramy*, *C. carpio*, and *O. niloticus* were 65.85%, 61.68%, and 55.63%, respectively. Heavy metal concentrations (mg/kg) in *O. goramy*: Pb (185) > Cu (83) > Cr (<5) > Ag (<14.66) > Hg and AS (0.43); *C. carpio*: Pb (152) > Cu (121) > Cr (50) > Ag (15) > Hg and AS (0.46 and 0.50); *O. niloticus*: Cu (160) > Pb (149) > Cr (50) > Ag (15) > Hg and As (0.53 and 0.50).

Keywords: Aquaculture, Fish scale waste, scale yield, heavy metal

1 Introduction

Aquaculture is experiencing rapid development as an emerging and indispensable sector in efforts to produce animal-based foods on a global scale [1-3]. The growth of aquaculture globally has made a positive contribution to global food and nutrition security, increased fish supplies worldwide, and reduced the impact of reduced fish production from capture fisheries to meet the increasing demand for fish [4-6].

In Indonesia, there are three main sources of fish supply: production from fish farming and fishing in inland waters, brackish water, and sea. The total aquaculture production in Indonesia reached 16,032,122 metric tons (mt). The details are 3,374,924 mt (21.05%) of freshwater aquaculture production, 9,884,670 mt (61.65%) of seawater aquaculture production, and 2,772,568 mt (17.29%) of brackish water aquaculture production. [7].

Over the past decade, there have been significant developments in several types of freshwater aquaculture commodities, including tilapia, Clarias catfish, Pangasius catfish, carp, and gourami. Together, these five types of fish contributed 37.39%, 33.35%, 12.38%, 9.28%, and 6.98% to the total freshwater aquaculture production [7]. Development of scaly fish cultivation, such as giant gourami, carp, and tilapia has also been carried out in West Sumatra Province, including the cultivation of these three species in floating net cages on Lake Maninjau [8,9].

However, every year it faces the problem of mass mortality of farmed fish in Lake Maninjau due to poor water quality [10]. The scales of these three farmed fish have not been utilized as a food source for both humans and animals, so they become a burden of waste that enters the lake's water bodies. Therefore, it is essential to analyze the heavy metal content in the fish scales of these three farmed fish *Osphronemus (O) gourami*, *Common (C) carp*, and *Oreochromis (O) niloticus*.

2 Material and methods of research

2.1. Biometric parameter of three farmed fish samples

A total of 10 fish samples, consisting of *O. guromy*, *C. carpio*, and *O. niloticus*, were collected from a floating net cage culture in Maninjau Lake. Upon reaching the laboratory, each fish species underwent individual weighing (referred to as TW), and their standard length (SL) and maximum height (H) were assessed. The standard length was measured from the mouth to the tip of the upper lobe of the caudal fin, while height was measured vertically, excluding the fins. The condition factor (CF) was also calculated using the formula CF = (TW/SL^3) x 100.

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2.2. Fish Scale Preparation

A comprehensive washing process was carried out for every 1,000 grams of wet scales from *O. goramy, C. carpio*, and *O. niloticus*. The fish scales underwent thorough washing in a 10% w/v NaCl solution, with a solution-to-scale ratio of 1:10 (w/w). This washing procedure lasted for 24 hours, maintaining a temperature of 4ºC. The washing process is repeated every eight hours to remove useless protein. This frequent repetition is done to ensure the complete removal of foreign protein present on the fish scales. The recurrent washing intervals contributed to a heightened level of purity in the fish scales, effectively preparing them for subsequent processing steps.

Moreover, the scales of *O. goramy*, *C. carpio*, and *O. niloticus* underwent a three-step washing process in low mineral water at room temperature for 10 minutes per cycle, followed by drainage. Subsequently, the scales fish were meticulously arranged within a cooker featuring pressure control and cooking time settings (specifically, a Pearl Pressure Cooker with a 5.5-liter capacity, manufactured in Indonesia). The heating procedure was initiated within the cooker, allowing the temperature to ascend to 121°C, accompanied by a pressure of 15 *psi*, as visually indicated by the temperature and pressure panel. At this juncture, the timer was configured for a duration of 10 minutes.

Subsequently, each batch of 100 grams of wet weight from the scales of *O. goramy*, *C. carpio*, and *O. niloticus* underwent a drying procedure using a 28L Stainless Steel Black Digimatic Oven Toaster set to 50°C for a duration of four hours until the moisture content reached 10%. Once dried, the fish scales were subjected to processing using a Miller Powder Grinder with a capacity of 100 grams, resulting in fine flour. This resultant flour was then sifted through a mesh size of 60 μm to facilitate the analysis of heavy metal content.

2..3. Yeild of reindeer and heavy metal analysis of fish scales

The outcomes derived from the fish scales were evaluated through the employment of the dry weight to wet weight formula multiplied by 100. Measurement of water absorption capacity (WAC) = (Wo-Wn)/Wo) × 100. Meanwhile, the presence of heavy metals, namely Hg, Pb, Cr, As, Ag, and Cu, underwent assessment using inductively coupled plasma mass spectrometry (specifically, the Optima 2000 DV model by PerkinElmer Inc., USA). This analytical approach adhered to the guidelines laid out in the Official Methods of Analysis of AOAC International, 19th Edition [11]

3 Result and discussion

3.1. Biometric parameter of three farmed fish samples

Table 1 showcases the average measurements for standard length, wet weight, width, and condition factor of the three fish species identified in Lake Maninjau. Significant statistical disparities were identified across the three fish species concerning standard length, wet weight, width, and condition factor ($p<0.05$; Table 1). In this study, the body widths of *O. goramy*, *C. carpio*, and *O. niloticus* based on standard length were 54.36%, 36.81%, and 39.71%, respectively. Similarly, as the report submitted by [12], the body width of *C. carpio* reached 31.71%, *Labeo rohita* 28.56%, and *O. mossambicus* 40.15%. Furthermore, the body width of these three species is more significant than that of sea bass (*Dicentrarchus labrax*), which has a percentage of 25.22% [13]. On the other hand, the condition factor values of *O. goramy*, *C. carpio*, and *O. niloticus* were 3.91, 3.99, and 3.61. This value indicates that these three fish species are in the plump category.

Table 1. Biometry of three species of farmed fish

	Species		
	O.goramy	*C. carpio*	*O. niloticus*
Biometric measurements			
Wet weight (g)	595.40 ± 32.31^{a}	633.30 ± 87^{b}	210.5 ± 12.12^{c}
Standard length (cm)	23.32 ± 0.62^{a}	26.01 ± 2.03^{b}	18.0 ± 0.53^{c}
Width (cm)	12.60 ± 0.36^{a}	9.90 ± 0.44^{b}	7.15 ± 0.21^{c}
Condition factor	3.91 ± 0.22^{a}	3.99 ± 0.65^{b}	3.61 ± 0.21^{c}

Mean ± SD (n = 10) with different letters in the same row a significantly different.

3.2. The yield of fish scales

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The results of drying fish scales after exposure to 50°C for 4 hours were recorded in Table 2. There were significant differences ($p < 0.05$; see Table 2) in net scale yields among the three fish species cultured in Lake Maninjau. Differences in fish scale production can be caused by factors such as scale type, scale thickness, fish age, and type of feed [14,15]. In addition, the method of drying and immersion of scales can also affect the weight of fish scales after the demineralization process [16-18].

Table 2. The fish scales' weight before and after the demineralization

Species	The fish scales' weight before the demineralization (g)	The fish scales' weight after the demineralization (g)	Water absorption capacity (%)	Yield (%)
O.goramy	100 ± 0.00	65.85 ± 1.10[a]	34.15 ± 1.10[a]	65.85 ± 1.10[a]
C. carpio	100 ± 0.00	61.68 ± 1.10[b]	38.32 ± 1.10[b]	61.68 ± 1.10[b]
O. niloticus	100 ± 0.00	55.63 ± 1.32[c]	44.37 ± 1.32[c]	55.63 ± 1.32[c]

Mean ± SD (n=3) with different letters in the same column significantly different.

3.3. Heavy metal content

Recently, the pollution problem of the aquatic environment has become severe and has spread worldwide, including in Indonesia. The industrial, agricultural, aquaculture and commercial chemical waste sectors are increasing waste discharge into the aquatic environment. The aquatic environment used for fish farming in rivers, lakes, and reservoirs is also affected, causing adverse effects on aquatic organisms, including fish [19]. According to Syandri et al. [20], Lake Maninjau receives wastewater discharge from agriculture, residents, and detergents. While in the water body, is farmed fish in floating net cages. Fish are often at the top of the aquatic food chain, meaning that metals accumulate at much higher concentrations than water and sediments [21].

This study observed heavy metals Hg, Cr, As, and Ag levels in fish scale flours, which showed no significant difference ($p > 0.05$). However, significant differences ($p < 0.05$) were seen in Pb and Cu content among various fish species (Table 3). All heavy metal content in fish scale flours remains below the threshold set by the Indonesian National Standard. This result is in line with the provisions of the Drug and Food Control Agency Regulation Number 9 of 2022, which regulates the Maximum Limit of Heavy Metal Contamination in Processed Food Products. Based on this regulation, the maximum limit for heavy metal contamination in processed fishery products is set at 5.0 μg/100 g for Hg, 20.0 μg/100 g for As, 3.0 μg/100 g for Pb, and 3.0 μg /100 g for Cd.

It turns out that the heavy metal content in fish scales is almost comparable to the content in fish meat. For example, in the fish, *Oxyeleotris marmorata* and *Rasbora lateristriata,* which live in Lake Maninjau, the Cuprum (Cu) content in the flesh of each fish are 148 and 128 μg/100 g. Nonetheless, the Pb content is lower in fish meat were 9.3 and 2.67 μg/100 g [20].

Table 3: Heavy metal concentrations in fish scales flour

Heavy metal (μg/100 g)	Species			Limits (μg/100 g)	
	O.goramy	*C. carpio*	*O. niloticus*	Indonesian National Standard	WHO Guidelines
Mercury, Hg	0.43 ± 0.05	0.46 ± 0.05	0.53 ± 0.0.5	5.0	5.0
Lead, Pb	185.00 ± 1.00[a]	152.33 ± 0.57[b]	149.33 ± 1.52[c]	3.0	2.0
Chromium, Cr	53.33 ± 5.77	50.00 ±10.00	50.00 ±10.00	100	100
Arsenic, As	0.43 ± 0.05	0.50 ± 0.10	0.50 ±0.10	20.0	20
Silver, Ag	14.66 ± 0.57	14.66 ± 0.57	15.33 ± 0.57	5.0	5.0
Cuprum, Cu	83.00 ± 1.00[a]	121.33 ±1.52[b]	160.00 ±1.00[c]	2000	2000

Mean ± SD (n = 3) with different letters in the same row a significantly different.

Several types of fish have the ability to absorb heavy metals such as lead (Pb) from their environment [22,23]. Fish scales are one part of the body that can contain heavy metals and other substances from the aquatic environment where the fish live [21]. Fish scales are one place where these metals can accumulate over time. This is the reason why analysis of fish scales is sometimes used to monitor exposure levels to heavy metals in the aquatic environment. However, keep in mind that the ability of fish to absorb heavy metals and their impact depends on many factors, such as the type of fish, environmental conditions, level of exposure, and others [23,24].

4 Conclusions

The ability of water absorption and scale production of the three cultivated fish species showed differences. The average content of heavy metals Pb and Ag in the scale meal of the three cultivated fish species has exceeded the specified threshold for heavy metal contamination in processed food. However, there are also heavy metal contents such as Hg, Cr, As, and Cu which are still below the established quality standards. Accurate data on heavy metal content in fish scale meal from three local fish species in the study area is still limited. Therefore, the data regarding heavy metal content presented in this study will be the basis for future research on heavy metal content in various organs of the three fish species. This research has an important contribution in the aspect of providing food for humans and as feed for fish seeds.

Acknowledgements
The author's team sincerely thanks all participants of this scientific project for their assistance in conducting experimental research. We also thank the Institute for Research and Community Service, Universitas Bung Hatta for their assistance and support.

Financing
This study was conducted within the framework of the project No. 86/E5/PG.02.00.PL/2023 funded by the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia.

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