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Assessing the Levels of Heavy Metals of Lead (Pb), Mercury (Hg), and Cadmium (Cd) in Native Fishes of the Saimareh River and Farmed Fishes in Ilam Province, Western Iran

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ABSTRACT

Introduction: Heavy metals tend to accumulate in fish tissues, making fish effective biological indicators for environmental contamination. As fish occupy higher levels in the food chain and represent a key component of the human diet, monitoring their health is critical for food safety. This study aimed to evaluate the concentrations of lead (Pb), mercury (Hg), and cadmium (Cd) in native fish species from the Saimareh River and farmed rainbow trout in Ilam Province, western Iran.

Methods: A total of 30 randomly selected samples, including native fish from the Saimareh River and farmed rainbow trout from Ilam Province, were collected for analysis. Samples were washed with distilled water, dried at 105°C for 24 hours, and weighed. Heavy metal concentrations were measured in dry weight using a PG Instruments AA990F atomic absorption spectrophotometer. Data were analyzed using STATA version 18.

Results: In farmed rainbow trout, the mean concentrations of lead, mercury, and cadmium were 0.079, 0.033, and 0.037 mg/kg, respectively, with standard deviations of 0.05, 0.03, and 0.05. The average weight of these fish was approximately 1400 grams. In native fish, the mean concentrations of lead, mercury, and cadmium were 0.1, 0.023, and 0.013 mg/kg, respectively, with standard deviations of 0.03, 0.01, and 0.01. The average weight of native fish was about 1314 grams. The overall mean concentrations for lead, mercury, and cadmium across all samples were 0.089, 0.028, and 0.025 mg/kg, respectively, with an average weight of around 1350 grams. Overall, native fish had higher lead levels compared to farmed trout, while farmed trout showed higher concentrations of mercury and cadmium.

Conclusion: The concentrations of Pb, Hg, and Cd in both farmed and native rainbow trout from Ilam Province were within acceptable safety limits, posing no significant health risk to consumers. These findings support the safety of consuming these fish species from a heavy metal contamination perspective.

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Introduction

Human activities such as urbanization and agriculture introduce large amounts of wastewater containing heavy metals into aquatic ecosystems. Due to their toxicity and resistance, these metals accumulate in the fish body and are finally transmitted to humans, creating serious health risks for them (Loghmani et al., 2019; Chakeri et al., 2015). With the development of industries and growth of populations, this wastewater enters aquatic environments and accumulates in aquatic organisms. The absorption amount of these metals is influenced by the environmental conditions of the water (Ebrahimi et al., 2012; Samsampour et al., 2013; Dehghan et al., 2019). The consumption of seafood, especially fish, has increased due to its high protein and vitamin content. Fish contributes to an average of 47 grams per day to the global diet, while in Iran this amount is only 5 grams. Fish provides 20 percent of the world's animal protein, and its per capita consumption has doubled over the past 40 years (Mashroofeh et al., 2012; Bosch et al., 2016). Studies demonstrated that regular fish consumption can reduce the risk of heart attacks by half and is beneficial for neurological development and the reduction of psychological disorders (Sheikhzadeh and Hamidian, 2021). Heavy metals are among the most important environmental pollutants, accumulating in aquatic organisms and are considered as a serious threat to aquatic ecosystems and living beings (Behzadi and Roosta, 2025). The main sources of pollution include industrial waste and mining activities. These metals can cause severe health issues in humans, including damage to the liver, kidneys, and neurological disorders (Türkmen and Ciminli, 2007; Milenkovic et al., 2019; Fidan et al., 2008). Fish are exposed to heavy metals such as copper, zinc, cadmium, mercury, lead, and nickel, and their accumulation depends on several factors, including habitat and diet. Mercury, cadmium, arsenic, and lead create the greatest risks to human health.

A study by Tahsini et al. (2018) showed that the average concentrations of cadmium in the liver and muscle of rainbow trout were 0.62 and 0.16 micrograms per gram, respectively, and the concentrations of lead were 26.31 and 23.65 micrograms per gram of wet weight, which were higher than international permissible levels (Tahsini et al., 2018). Also, Tabibzadeh et al. (2015) investigated the accumulation of mercury, cadmium, and lead in various organs of rainbow trout and demonstrated that the levels of these metals increase with the age and growth of the fish. The concentrations of mercury, cadmium, and lead in the muscle of farmed trout were measured as 0.021 ± 0.002, 0.119 ± 0.022, and 0.214 ± 0.038 micrograms per gram, respectively (Tabibzadeh et al., 2015). Lead, cadmium, and mercury are heavy and toxic metals with hazardous effects on human health. Lead can lead to neurological damage, high blood pressure, and behavioral disorders. Cadmium is associated with severe bone pain and kidney damage, while mercury can cause vision and hearing problems and brain damage. These metals accumulate in fish tissues at varying levels; therefore, fish are a suitable biological indicator for assessing the concentration of heavy metals in aquatic environments. In addition, fish are at the top of the food chain and play a significant role in human diets, thus, ensuring their safety is essential for healthy nutrition (Tahsini et al., 2018). Therefore, the presence of heavy metal residues in fish meat and their harmful effects on human health is a major concern for food safety. Monitoring and controlling the level of contaminants in food and identifying sources of pollution can have a considerable impact on human health and longevity. No previous study has measured the concentration of heavy metals in native fish species of Ilam Province. Therefore, this study was designed to determine the levels of lead, mercury, and cadmium in native fish of the Seimareh River and farmed trout in Ilam Province.

Materials and Methods

The present study was a descriptive-analytical investigation, which was conducted after obtaining Ethics Code from the Ilam University of Medical Sciences and doing the necessary arrangements. The statistical population included native fish of the Seimareh River and farmed fish of Ilam Province, located in west of Iran.

Sample Size and Sampling Method

According to the sample sizes used in previous studies, 30 fish samples were collected from the fish available in the Seimareh River and farmed fish of the Ilam Province, each including 15 fish.

Data Collection Tools and Methods

The concentrations of heavy metals in the sampled fish were measured using the atomic absorption method in the laboratory, and the results were recorded in a pre-designed checklist. The checklist included details such as fish weight, fish type, and levels of lead, cadmium, and mercury.

The sampling method was completely random. Samples were collected in three rounds, each from five different locations. It was tried to select fish samples with similar weights and minimal differences in age and size. The, approximately one kilogram of each fish was separated as a sample and transported to the laboratory at the Medicinal Plants Research Center for analysis. The collected samples were digested using the wet digestion method to measure the concentrations of heavy metals (mercury, lead, cadmium).

After being washed with single-distilled water, the samples were dried, and then placed in an oven at the temperature of 105°C for 24 hours to completely remove moisture. The dry weight was recorded. The dried samples were ground, and 20 grams of each were placed in a porcelain crucible and heated in a furnace at the temperature of 500°C for 4 hours. Then, 10 milliliters of 65% nitric acid were added to 0.5 grams of the ash from each sample, and the mixture was placed in a laboratory water bath at 70°C to be completely digested. After cooling, the sample was filtered using Whatman No. 41 filter paper and brought to a final volume of 50 milliliters in a flask with triple-distilled water. All preparation, digestion, and analysis steps were performed according to standard methods. Standard solutions of heavy metals (Cd, Pb, and Hg) with a concentration of 1000 mg/L, along with the required acids, were obtained from Merck Company. Different concentrations of each heavy metal were prepared by diluting the standard solutions, and a standard curve was drawn. The concentrations of cadmium, lead, and mercury were determined using a PG Instruments atomic absorption spectrophotometer, AA990F model.

Data Analysis Method

STATA statistical software, version 18 was used for data analysis. To describe the data, frequency distribution tables and measures of mean and standard deviation for quantitative variables, along with bar charts, were used. For comparing the mean concentrations of heavy metals with international standards, non-parametric tests such as the Bootstrap t-test and Permutation t-test were employed. Additionally, linear regression was used to examine the relationship between fish weight and the concentration of heavy metal. A significance level of 0.05 was considered for all comparisons.

Results

The present study was conducted to determine the levels of heavy metals—lead, mercury, and cadmium—in native fish of the Seimreh River and farmed fish of Ilam Province. The results of the study are presented in two sections (descriptive and analytical findings). The subjects are described in the descriptive findings section. The analytical findings section includes three parts; part one compares the levels of the examined metals with global standards, part two compares them with Iran's national standards, and part three examines the relationship between the metal concentrations and fish weight.

Descriptive Findings

In this study, 30 fish samples (15 samples each from farmed rainbow trout and native fish of the Seimareh River in Ilam Province) were analyzed for heavy metals: mercury, lead, and cadmium. The results demonstrated that the lowest and highest lead concentrations in farmed rainbow trout were 0.007 and 0.19 micrograms per gram of dry weight, with a mean of 0.079 micrograms per gram. For native fish, the lowest and highest lead concentrations were 0.015 and 0.16 micrograms per gram of dry weight, with a mean of 0.1 micrograms per gram. According to the above results, the lead concentration in rainbow trout was lower compared to the native fish. The mean mercury and cadmium levels in farmed rainbow trout were 0.033 and 0.037 micrograms per gram, respectively. In native fish, the mean mercury and cadmium levels were 0.023 and 0.013 micrograms per gram, respectively. The mean mercury and cadmium levels in farmed rainbow trout were higher compared to the native fish of Ilam Province. The mean weight of the rainbow trout was 1,400 grams, while the mean weight of the native fish was 1,314 grams (Table 1).

Table 1. Descriptive information on measurements of lead, mercury, cadmium, and weight in the studied fish

Variable	Mean ± SD	Minimum	Maximum	Count
Farmed Rainbow Trout				15
Lead	0.079 ± 0.05	0.007	0.19	
Mercury	0.033 ± 0.03	0.006	0.14	
Cadmium	0.037 ± 0.05	0.002	0.15	
Weight	1400.22 ± 667.86	1080	1900	
Native Fish				15
Lead	0.1 ± 0.03	0.015	0.16	
Mercury	0.023 ± 0.01	0.008	0.04	
Cadmium	0.013 ± 0.01	0.001	0.04	
Weight	1314.23 ± 667.66	1090	1800	
Total				30
Lead	0.089 ± 0.05	0.007	0.19	
Mercury	0.028 ± 0.02	0.006	0.14	
Cadmium	0.025 ± 0.03	0.001	0.15	
Weight	1350.23 ± 667.94	1080	1900	

Based on the results of the Table 2, the measured levels of lead, cadmium, and mercury in the studied fish (farmed rainbow trout and native fish of Ilam Province) were below the permissible limits set by the World Health Organization. Additionally, the levels of lead, cadmium, and mercury were also lower than the permissible limits established by the FDA, the UK Ministry of Agriculture, Fisheries, and Food, and the National Health and Medical Research Council of Australia.

Table 2. Comparison of the results of current study with international threshold limits

Standards	Heavy Metals		
	Mercury (µg/g)	Cadmium (µg/g)	Lead (µg/g)
World Health Organization (WHO)	0.5	0.2	0.5
U.S. Food and Drug Administration (FDA)	0.1-0.5	0.1	0.5
Australian National Health and Medical Research Council	1.0	0.05	1.5
UK Ministry of Agriculture, Fisheries, and Food (UKMAFF)	0.5	0.02	0.2
Farmed Rainbow Trout	0.033	0.037	0.079
Native Fish from Ilam Province	0.023	0.013	0.1

Comparing the concentrations of heavy metal with the permissible limits declared by Iran's national standards was conducted, and the results are presented in table 3. The absorption levels of cadmium, lead, and mercury in the studied fish were generally below the permissible limits established by Iran's national standards, with the lead concentration showing a statistically significant difference from the permissible limit ($P = 0.0006$). Also, the overall concentration of mercury in all fish was found to be 0.028 micrograms per gram, which is lower than the national standard of 0.5 micrograms per gram, showing a statistically significant relationship ($P < 0.001$).

According to the results of table 3, the mercury levels in both farmed rainbow trout and native fish of Ilam Province were below the permissible limit of ISNO12968, and significant differences was observed in both groups of fish ($P < 0.001$). The concentration of leaf in native fish of Ilam Province and farmed rainbow trout were also below the permissible limits set by Iran's national standards, showing a statistically significant difference ($P < 0.05$). In terms of cadmium levels, both in farmed rainbow trout and native fish of Ilam Province, the concentrations were below the permissible limit, but no statistically significant difference was found ($P > 0.05$).

Table 3. Comparison of the results of the current study with the national standard thresholds of Iran

Iran National Standard (ISNO12968)	Heavy Metals			P-value
	Mercury (µg/g)	Cadmium (µg/g)	Lead (µg/g)	
Standard Threshold	0.5	0.1	0.3	
Farmed Rainbow Trout	0.033	<0.001	0.037	0.208
Native Fish from Ilam Province	0.023	<0.001	0.013	0.133
All Fish	0.028	<0.001	0.025	0.088

Considering the estimated linear regression formula, the amount of Pb in the studied fish was calculated based on weight, which showed a statistically significant difference between the weight and lead concentration in farmed rainbow trout:

$$Pb = 0.00015 \times [(\text{weight of the fish}) - 0.12]$$

According to Figure 1, the concentration of lead increasing with increasing the weight of the farmed rainbow trout increases, based on the above equation.

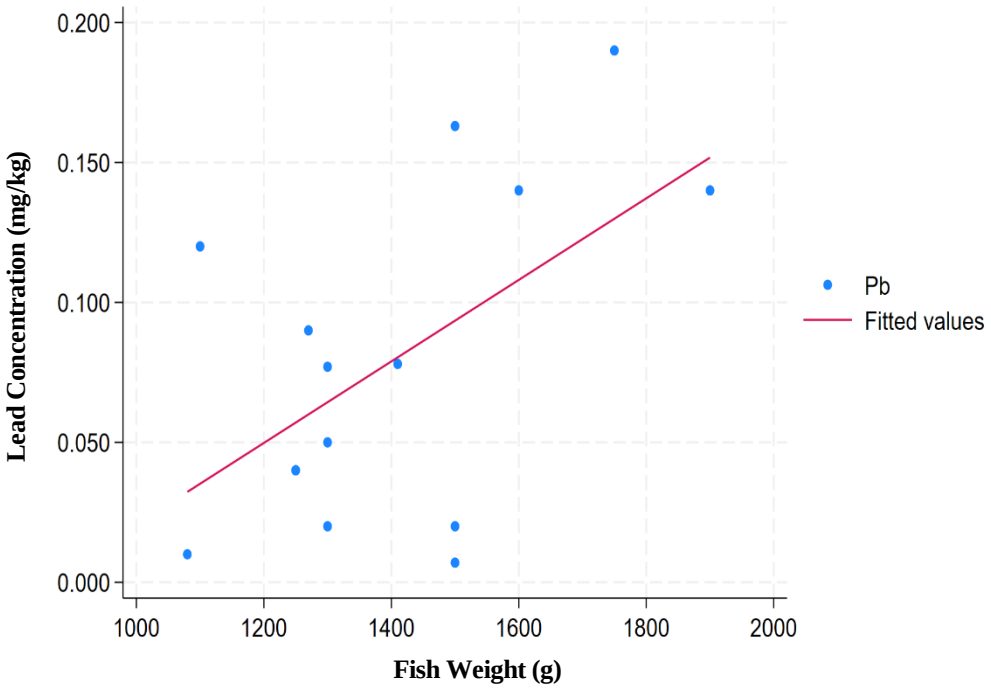

Figure 1. Regression Line of Lead Levels in Farmed Rainbow Trout in Relation to Fish Weight

Discussion

Measuring the concentrations of heavy metals in aquatic organisms is of a great importance due to managing aquatic ecosystems and ensuring food safety for humans. Considering that farmed rainbow trout and native fish of the Seimareh River in Ilam Province are edible fish and are caught by local people for consumption, ad also, the significance of heavy metal accumulation in fish and the harmful effects of these pollutants on health, this research was conducted so to determine the levels of heavy metals: lead, mercury, and cadmium in native fish from the Seimareh River and farmed fish of Ilam Province in west of Iran.

In the present study, the amount of lead in farmed rainbow trout was higher than cadmium, and cadmium was higher than mercury, which is consistent with the findings of the study of Dehghan et al. In the study of Dehghan et al., the accumulation of heavy metals in the muscle of sea bass was reported as follows: lead was higher than cadmium, and cadmium was higher than mercury (Samsampour et al., 2013). Additionally, in the native fish of Ilam Province, the concentration of lead was higher than mercury, and mercury was higher than cadmium. In Dehghan et al.'s study, the levels of heavy metals in the muscle of mullet and tilapia were also reported as follows: cadmium was higher than lead, and lead was higher than mercury (Samsampour et al., 2013). The low accumulation of heavy metals in muscle tissue may be due to the low levels of metal-binding proteins. It seems that muscle tissue is not considered a major place for the accumulation of heavy metals (Roméo et al., 1999).

The results of the present study demonstrated that the concentration of mercury in farmed rainbow trout ranged from 0.006 to 0.14 µg/g. In native fish of Ilam Province, the concentration of mercury was between 0.04 and 0.008 µg/g. Also, the findings of Roméo et al. demonstrated that the concentration of mercury in the edible tissues of pelagic fish (0.03 to 0.09 µg/g) is lower than that in demersal fish (0.12 to 0.42 µg/g) (Roméo et al., 1999). Moreover, the differences in the accumulation of elements among different species are influenced by dietary behaviors (Watanabe et al., 2003), age, size, and length of the fish (Al-Yousuf et al., 2000; Solgi E, Yaghobifar et al., 2016), habitat, environmental conditions (Canli and Atli, 2003), as well as the physical and chemical properties of the environment, such as water hardness, pH, temperature, nutrients, and fish growth time (Pakzad, 2013).

The concentration of cadmium in the present study was found to be between 0.15 and 0.002 µg/g in farmed rainbow trout, while the

concentration of cadmium in native fish of Ilam Province ranged from 0.001 to 0.04 µg/g. In the study of Dehghan et al.'s, the concentrations of cadmium in the muscle of sea bass, mullet, and tilapia were reported to be between 1.85 and 0.21 µg/kg wet weight (Samsampour et al., 2013). The average concentration of cadmium in farmed rainbow trout was 0.037 ± 0.05 µg/g, and it was 0.013 ± 0.01 µg/g in native fish of Ilam Province, which demonstrates that the concentration was higher in farmed rainbow trout compared to in native fish. In the study of Dehghani and Farzin, which investigated the concentration of cadmium in mullet caught from the Persian Gulf, the average concentration of cadmium was reported to be 0.63 ± 0.29 µg/g (Dehghan Tarzjani, 2015), which is higher than the concentration of cadmium found in the present study.

The results of the present study demonstrated that the concentrations of lead, cadmium, and mercury measured in farmed rainbow trout and native fish of Ilam Province were below the permissible limits set by the World Health Organization and the FDA, which is consistent with the findings of Dural et al. In the study of Dural et al.'s, the accumulation of heavy metals in different tissues, including the muscle of fish caught from Kamiliak Lagoon Mugil cephalus, Dicentrarchus labrax, Sparus aurata (MCDLSA) in Turkey, showed that lead, nickel, manganese, and cadmium had the lowest accumulation in muscle tissue, and all metal concentrations were below the permissible levels declared by FAO standards (Dural et al., 2007). In the study of Rastiannasab et al., the results demonstrated that the concentrations of heavy metals, including mercury, cadmium, and lead, in the muscle of farmed rainbow trout from the banks of the Bashar River were lower than the permissible limits set by global standards, including FAO, EC, MAFF, and FDA. Based on the findings of this study, farmed fish from the banks of the Bashar River are currently safe for human consumption and free from heavy metal contamination, which are in line with the results of the present study (Rastiannasab, 2021).

In the study of Tahsini et al., the comparison of the measured concentrations of lead and cadmium in the muscle tissue of rainbow trout with international permissible standards demonstrated that the lead concentration exceeded the standards set by the World Health Organization, the Food and Agriculture Organization, the UK Department for Environment, Food & Rural Affairs, and the United States Environmental Protection Agency. The higher level of lead compared to the mentioned standards could be considered a warning, which might be due to the quality of the water from the rivers that feed the fish ponds, as well as the additives used in the ponds, which were not in line with the results of the present study (Tahsini et al., 2018). Also, in the study of Tahsini et al., the concentration of cadmium was lower than the standards declared by the World Health Organization, the Food and Agriculture Organization, the UK Department for Environment, Food & Rural Affairs, and the United States Environmental Protection Agency, which aligns with the results of the present study, where the cadmium concentration was below the standards set by the World Health Organization, national standards of Iran, and etc. (Tahsini et al., 2018).

In the present study, the concentration of lead in farmed rainbow trout and native fish of Ilam Province was lower than the standards set by the World Health Organization, FDA, National Health and Medical Research Council of Australia, the UK Department for Environment, Food and Rural Affairs, and the national standards of Iran. In the study by Roomiani and Sharifpour, the concentration of lead in the tissues of the studied fish was reported to be below the international standard, which is in line with the results of the present study (Roomiani et al., 2014). Moreover, in the study of Yilmaz and Dogan, which was conducted on some heavy metals in the liver, muscle, and gills of tilapia fish in Koycegiz Lake, the lowest concentrations of lead, zinc, and cadmium were observed in the edible part (muscle) of tilapia fish. Moreover, the concentrations of lead and zinc in tilapia were higher than the amounts recommended by WHO for human consumption, which were not in line with the results of the present study (Yilmaz and Doğan, 2008).

In the preset study, the concentration of mercury in farmed rainbow trout and native fish of Ilam Province was also below the standards declared by the World Health Organization, FDA, National Health and Medical Research Council of Australia, the UK Department for Environment, Food and Rural Affairs, and the national standards of Iran. In the study of Dehghan et al., the concentration of mercury in all three types of fish (Tilapia, catfish, and grouper) was lower than the standards of FDA, FAO, WHO, and the national standards of Iran. Sadeghi Rad et al. also determined that the mercury levels in the muscle and caviar of two species of Iranian sturgeon were lower than the global standards for human consumption (Sadeghi

et al., 2005). Owing to the fact that the concentrations of mercury in farmed rainbow trout and native fish of Ilam Province were below the global standards in this study and that sampling was conducted in the winter season, it can be justified that the concentration of mercury is lower in rainy seasons due to the dilution of pollutants.

In the present study, the concentration of cadmium in farmed rainbow trout and native fish of Ilam Province was lower than the standards set by the World Health Organization, FDA, National Health and Medical Research Council of Australia, the UK Department for Environment, Food and Rural Affairs, and the national standards of Iran. In the study by Tahsini et al., a cadmium concentration of 0.16 was obtained, which was lower than the international standards and it was in line with the findings of the present study (Tahsini et al., 2018). Additionally, in the study conducted by Dadelahi et al., the average concentrations of heavy metals, lead and cadmium, in the muscle tissue of the Persian catfish in the Arvandrud River were higher than the global standards, which were not in line with the results of the present study (Dadelahi et al., 2009). In the study of Pazira et al., the average concentration of lead in the muscle tissue of the Persian catfish was determined as 0.349 ± 0.173. Moreover, in the preset study, the concentration of lead in the muscle tissue of the Persian catfish was above the permissible limit of the WHO and FAO standards, which were not in lie with the results of the present study (Pazira et al., 2016).

The results of the preset study demonstrated that there was a statistically significant difference between the weight of the rainbow trout and the level of lead. According to the linear regression graph, the amount of lead increased with increasing the weight of the fish. Yousef et al. reported a strong positive and significant relationship between the body weight of Wallago attu fish in Punjab, Pakistan, and a range of heavy metals, including lead ($r = 0.928$) (Yousef et al., 2012). Oguzie reported a significant positive correlation between the body weight of fish and the concentrations of lead and other heavy metals in one of the rivers in Nigeria, which is in line with the results of the present study (Ekejiuba Oguzie et al., 2018). In terms of the concentration of lead in Qom fish, there was no relationship between the body weight of the fish and the accumulation of heavy metals, mercury and lead, which were not in line with the results of the present study; however, in terms of the concentration of mercury, the results were consistent with the preset study, which found no significant correlation between the body weight of the fish and mercury levels (Dehghan Tarzjani et al., 2015).

Conclusion

In the present study, the average concentrations of mercury, lead, and cadmium for farmed and native rainbow trout of Ilam Province were lower than the standards set by FAO, WHO, and the national standards of Iran and ind terms of nutrition, the presence of mercury, lead, and cadmium in these two types of fish does not pose a health risk to consumers. This is indicative of the fact that individuals consuming these fish are not at significant health risks caused by lead, cadmium, and mercury. It is recommended that the native fish of the Seimareh River in Ilam Province be compared with other types of fish. Future studies should scrutinize a broader range of heavy metals. Additionally, it is suggested that future studies consider variables such as the age and gender of the fish to investigate their relationship with the concentrations of metals.

Limitations and Challenges of the Research

Difficulty in obtaining various samples of native fish from the Seimareh River: The researcher sought help from Local fishermen.

Weight and age differences among the different fish caught: Only fish with similar weights, without significant differences, were selected for the study to ensure comparable results.

Declarations

Conflict of Interest

The authors declare no conflict of interest.

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Consent for Publications

All authors have read and approved the manuscript for publication.

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Authors' Contributions

DV, HGH, MB, EA, and HS contributed to the conceptualization of the study. DV and HGH were responsible for data curation, formal analysis, investigation, methodology, validation, and visualization. MB and EA supervised the project and handled its administration. HS also contributed to investigation and participated in writing the original draft as well as reviewing and editing the manuscript. DV, HGH, and HS were involved in drafting the original manuscript and its critical revision.

Ethical Considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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