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BIOACCUMULATION OF HEAVY METALS IN FRESHWATER FISH: CONSERVATION CONCERNS AT TAMAN RIMBA ILMU TANAH BRIS (TRIBE) UniSZA

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Abstract

Bioaccumulation refers to the process by which chemical substances accumulate within the tissues of living organisms over time, often resulting in concentrations higher than those found in the surrounding environment. This study aims to assess the concentration of heavy metals in water and fish samples collected from Taman Rimba Ilmu Tanah BRIS (TRIBE), Universiti Sultan Zainal Abidin (UniSZA). Sampling was conducted from November 2022 to January 2023, with both water and freshwater fish samples manually collected. The samples were analyzed using the acid digestion method (AOAC, 2016) and heavy metal concentrations were determined via Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES). The heavy metals detected in water samples were copper (Cu) (0.617ppm), iron (Fe) (0.165ppm), manganese (Mn) (0.161ppm) and aluminum (Al) (0.075ppm), in descending order of concentration. The concentrations of Al, Cu, Fe and Mn in water samples from all streams remained within the permissible limits established by the Department of Environment (DOE, 2017). In fish samples, the highest heavy metal accumulation was observed in Osteochilus vittatus, with Fe (1.683ppm) being the most abundant, followed by Al (0.484ppm), Mn (0.287ppm) and Cu (0.081ppm). However, all heavy metal concentrations in fish samples were below the permissible limits set by the Malaysian Food Act (MFA, 1985) and the Food and Agriculture Organization (FAO, 1983). Statistical analysis using ANOVA indicated a significant difference ($p < 0.05$) in Fe concentrations among fish samples collected from different streams. Water quality classification revealed that station 1 falls within Class II, suitable for recreational activities, whereas stations 2 and 3 are categorized as Class III, indicating the need for extensive treatment before use.

Keywords: *Freshwater fish; Heavy metals; Bioaccumulation,; TRIBE*

Introduction

Heavy metals are widely recognized for their toxicity, persistence in the environment and propensity to accumulate in aquatic organisms through bioaccumulation and bioconcentration. While some heavy metals occur naturally, others originate from anthropogenic activities [1]. Due to their hazardous nature, heavy metals pose a substantial

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threat to both the environment and human health. They are introduced into ecosystems through various waste streams, including liquid and solid waste and can enter aquatic environments via precipitation and surface runoff. Once released, heavy metals interact with environmental components such as soil, water and air, potentially increasing their toxicity and ecological impact.

Living organisms, including humans, are exposed to heavy metals through the food chain, primarily via bioaccumulation—the gradual accumulation of substances in the tissues of organisms over time. This process can occur through the ingestion of contaminated food or water, dermal absorption, or inhalation of polluted air. In aquatic ecosystems, heavy metals are introduced from various sources, including sewage discharge, pesticide runoff and recreational activities. The extent of heavy metal accumulation in these environments is influenced by several factors, such as oxygen levels, water hardness, pH, alkalinity and temperature [2]. Additionally, the size and weight of fish play a crucial role in determining the extent of heavy metal toxicity [3]. Bioaccumulation can have severe biological consequences, including developmental abnormalities, reproductive disorders and mortality. In some cases, bioaccumulated heavy metals undergo biomagnification, leading to increased concentrations as they move up the food chain [4].

Bioconcentration is another significant process by which chemical substances accumulate in the tissues of organisms at concentrations exceeding those found in their surrounding environment. This phenomenon is particularly relevant in aquatic ecosystems, where organisms directly absorb contaminants from water through their gills, skin, or ingestion of food [5]. The degree of bioconcentration is influenced by factors such as the physicochemical properties of the substance, organismal traits and environmental conditions. Chemicals that are resistant to degradation or possess long half-lives exhibit higher bioconcentration potential. Additionally, factors such as pH and ionization play a critical role in this process [4]. Elevated levels of heavy metals pose a serious environmental and public health concern, particularly through the consumption of contaminated freshwater fish. Consequently, freshwater fish species are frequently utilized as bio-indicators to monitor heavy metal pollution in aquatic environments.

The UniSZA Besut Campus established Taman Rimba Ilmu Tanah BRIS (TRIBE) as an educational and research center aimed at promoting sustainable environmental practices. TRIBE is part of the Green Campus initiative, which focuses on the sustainable development of the UniSZA Besut Campus [6]. The area is characterized by Beach Ridges Interspersed with Swales (BRIS) soils, which are predominantly sandy with a sandy loam texture, high sand content and an unstructured nature. BRIS soils also feature a hardened layer, limited water retention capacity and an acidic pH ranging from 4.3 to 5.8 [7]. As a Green Campus initiative, TRIBE integrates environmentally friendly practices and education to foster a sustainable ecological framework. Such initiatives provide institutions with opportunities to redefine environmental consciousness and establish innovative paradigms for addressing environmental, social and economic challenges. Within this ecosystem, fish play a vital role in the food chain, serving as essential components of aquatic biodiversity. However, the unique characteristics of BRIS soils can significantly influence water quality. Their sandy and unstructured composition, coupled with acidic pH levels, poses a risk of groundwater contamination. Additionally, the high susceptibility of BRIS soils to erosion, particularly in coastal areas, can lead to sedimentation in water bodies, thereby degrading water quality and adversely affecting aquatic life [7]. While trace amounts of essential metals are necessary for biological growth, excessive concentrations can be detrimental to living organisms. This study serves as a preliminary investigation into the bioaccumulation of heavy metals in freshwater fish within the TRIBE watershed. The findings provide valuable baseline data for researchers studying the TRIBE ecosystem and contribute to conservation efforts aimed at preserving this critical environmental component.

Materials and Methods

Study area

The study was conducted within the watershed of Taman Rimba Ilmu Tanah BRIS (TRIBE) at Universiti Sultan Zainal Abidin (UniSZA) (Fig. 1).

Fig. 1. Taman Rimba Ilmu Tanah BRIS (TRIBE) at UniSZA

The total area of the watershed at TRIBE, located on UniSZA's Besut Campus, encompasses approximately 20 hectares. Water and fish samples were collected from three designated sampling stations (Figs. 2 and 3) during November 2022 and January 2023.

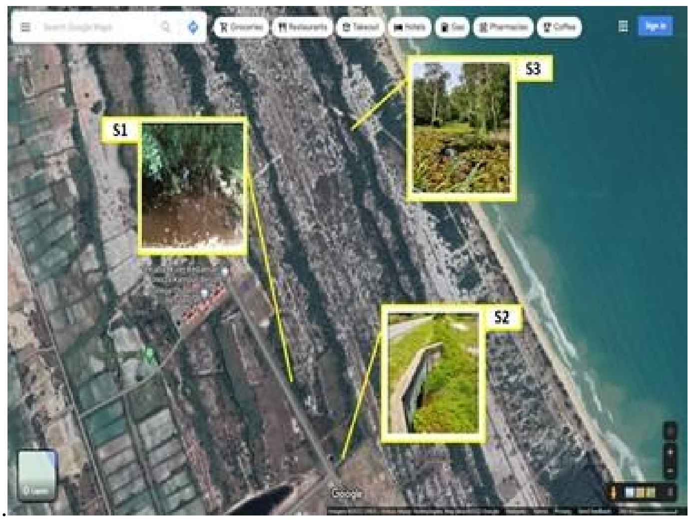

Fig. 2. The Location of sampling stations at TRIBE UniSZA Besut Campus

Fig. 3. Location of each station (Table 1)

Table 1. Coordinates of each station

Stations (S)	S1	S2	S3
Coordinates	05° 45' 37.7" N 102° 38' 04.9" E	05° 45' 39.9" N 102° 38' 02.6" E	05° 46' 03.7" N 102°38'17.9" E

Fish collection

The fish were caught from Taman Rimba Ilmu Tanah BRIS (TRIBE) watershed using the fishing net and placing fish in polyethylene bags. The collected fish samples were immediately stored in an ice box and transported to the laboratory. Upon arrival, the fish were identified and placed in polyethylene bags, which were then appropriately labeled. The samples were subsequently deep-frozen in a refrigerator until further analysis refering EPA, 1978.

Length and weight measurements

The fish samples were thawed at room temperature. Each sample's length (cm) and weight (g) were measured using a steel ruler and analytical balance. Then, the data was recorded [8].

Acid digestion method

Fish tissue samples were subjected to acid digestion following the method described in [9]. Each sample was placed in a test tube containing 10mL of 69% nitric acid and left at room temperature overnight in a fume hood. The following day, the samples were transferred to digestion tubes and any residue remaining on the test tube walls was rinsed with an additional 5mL of 69% nitric acid. The samples were then digested using microwave digestion for approximately 1.5 hours. Subsequently, 2mL of 30% hydrogen peroxide was added to each sample before allowing them to cool for 30 minutes in the fume hood. The resulting solution was filtered using 0.5mm filter paper and transferred into a 25mL volumetric flask. Deionized water was then added to the flask to bring the final volume to 50mL. The heavy metal content in the fish tissue was determined by analyzing the sample solution using Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES).

Metal calculation

The concentration of heavy metals in fish tissues was calculated by using the formula below [10]:

$$C = A \times B / M, \tag{1}$$

where: C = Concentration of test samples (ppm); A = Reading from ICP (ppm); B = Volume of final solution (mL) and M = Weight of sample (g)

Statistical analysis

Statistical analysis was performed using Microsoft Excel. A one-way Analysis of Variance (ANOVA) was conducted to assess the significant differences in heavy metal concentrations among freshwater fish samples. A p-value of ≤ 0.05 was considered statistically significant [11].

Results and Discussion

Heavy metals concentration in water samples

Table 2 indicates that copper (Cu) exhibited the highest concentration in water samples, ranging from 0.011 to 0.5792ppm. According to [12], elevated Cu levels in aquatic ecosystems are often associated with agricultural activities involving copper-based pesticides, fungicides and algaecides. Additionally, natural processes such as decaying vegetation, surface runoff, windblown dust and forest fires can contribute to increased Cu concentrations in water. Among the sampling stations, the highest mean Cu concentration was recorded at S3, likely due to stagnant water conditions that facilitate the accumulation of pollutants over time. However, Cu levels at all stations remained within permissible limits.

In contrast, iron (Fe) exhibited the lowest concentration among the analyzed heavy metals, ranging from 0.009 to 0.028ppm in water samples. The highest Fe concentration was observed at S3 (0.0284ppm). This finding suggests that the TRIBE area is minimally influenced by rock and soil weathering, urban storm runoff, industrial discharges and agricultural runoff [11]. The Fe concentrations were below the permissible limits set by [13] (0.2ppm) and [14] (1.0ppm).

Additionally, aluminum (Al) and manganese (Mn) concentrations in TRIBE water samples were slightly elevated, ranging from 0.008 to 0.04ppm and 0.007 to 0.038ppm, respectively. However, their levels remained below the permissible limits established by [13] and [14].

The findings of the present study were compared with those of *A.N. Adilah and H.N. Nadia* [13] in Chini Lake. The Cu (0.035ppm) and Mn (0.080ppm) concentrations in Chini Lake were similar to those recorded in the present study. However, the Fe concentration in the current study (1.57ppm) was lower than that reported in [13]. Heavy metal concentrations in water can vary between locations due to site-specific environmental factors. According to [13], the higher levels of heavy metals in Chini Lake were attributed to increased land-use activities in the area, while Sembilang River was affected by household and industrial discharges.

Heavy metals concentration in freshwater fishes

A total of 9 adult fishes were caught from TRIBE, comprising three species. The distribution of captured fish species includes Cyprinidae family which were *Rasbora trilineata* and *Osteochilus vittatus* and Channidae family *Trichopodus trichopterus*. The findings regarding measurements of freshwater fishes collected from TRIBE are presented Table 3. The three local species found along TRIBE showed dense distribution and share the same physical properties, including feeding behavior and habitats.

Table 4 presents the mean concentrations of aluminum (Al), iron (Fe), copper (Cu) and manganese (Mn) in the muscle tissue of the collected fish species. According to the results, *Osteochilus vittatus* exhibited the highest accumulation of Al (0.484 ± 0.459ppm) compared to *Rasbora trilineata* (0.243 ± 0.106ppm) and *Trichogaster trichopterus* (0.291 ± 0.126ppm).

Aluminum is known to be toxic to both terrestrial and aquatic organisms; therefore, elevated concentrations of Al can pose significant environmental concerns.

The Cu concentration in fish species ranged from 0.07 to 0.08ppm, with *O. vittatus* showing the highest mean Cu concentration (0.0805ppm), followed by *R. trilineata* (0.0725ppm) and *T. trichopterus* (0.0705ppm). Certain fish species, as well as many invertebrates, are highly sensitive to copper toxicity.

Table 2. The mean concentration of heavy metals (ppm) ± SD in freshwater collected from TRIBE

Stations (S)	Aluminium (Al)	Copper (Cu)	Iron (Fe)	Manganese (Mn)
S1	0.0087 ± 0.0001	0.011 ± 0.00011	0.0092 ± 0.0001	0.00755± 0.0001
S2	0.0260 ± 0.0039	0.0370 ± 0.0006	0.018 ± 0.00056	0.0266 ± 0.00075
S3	0.0416 ± 0.0030	0.5792 ± 0.0098	0.0284 ± 0.0011	0.0389 ± 0.00072
Previous study (ppm				
A.N. Adilah and H.N. Nadia [13]	-	0.035	1.57	0.080
T.N.B.T. Ibrahim et al., [14]	0.007	0.027	3.01	0.068
Permissible limit (ppm)				
Department of Environment (DOE) [15]	10.0	0.20	1.0	0.20
World of Health Organization (WHO) [16]	0.2	1.0	0.3	0.05

Table 3. List of local fishes caught from TRIBE

Species	Length (cm)	Weight (g)
Rasbora trilineata	5.0 – 7.0	0.5 – 1.0
Trichopodus trichopterus	5.0 – 12.0	0.4 – 7.0
Osteochilus vittatus	5.0 – 10.0	0.4 – 5.0

Table 4. The mean concentration of heavy metals (ppm) ± SD in individual species collected from TRIBE

Species	Al	Cu	Fe	Mn
Rasbora trilineata	0.243 ± 0.106	0.0725 ± 0.0245	1.183 ± 0.852	0.00929 ± 0.00167
Trichopodus trichopterus	0.291 ± 0.126	0.0705 ± 0.0475	1.834 ± 1.490	0.172 ± 0.206
Osteochilus vittatus	0.484 ± 0.459	0.0805 ± 0.0569	1.683 ± 0.764	0.287 ± 0.238
Permissible limit (ppm)				
Organizations	**Al**	**Cu**	**Fe**	**Mn**
Malaysian Food and Regulation (MFR) [17]	-	30	0.3	-
Food and Agriculture Organization (FAO) [18]	-	10	43	1.0
Previous study (ppm)				
M.A. Ahsraf et al., 2012 [19]	-	0.82	1.39	-
M.A. Salam et al., 2020 [20]	-	0.013	0.038	-
M. Stancheva et al., 2014 [21]	-	0.34 – 1.40	2.2 – 9.0	0.06 – 0.17
J.M. Renata et al., 2012 [22]	-	1.20 – 2.90	1.60 – 7.50	0.30 – 1.70

The Fe concentrations in *R. trilineata* (1.183 ± 0.852ppm), *T. trichopterus* (1.834 ± 1.490ppm) and *O. vittatus* (1.683 ± 0.764ppm) were analyzed and statistical analysis indicated a significant difference in Fe levels among the sampling stations ($p < 0.05$, $p = 0.001$). Despite these variations, Fe levels in all three fish species remained below the permissible limits set by [17]. Iron is an essential mineral for fish, playing a vital role in oxygen transport, DNA synthesis and energy production. However, excessive Fe accumulation may be harmful to both aquatic organisms and human health if consumed in large quantities.

Mn concentrations in fish samples from the three streams were 0.00929 ± 0.00167ppm, 0.172 ± 0.206ppm and 0.287 ± 0.238ppm, respectively. *R. trilineata* exhibited the lowest Mn concentration, while *O. vittatus* had the highest. Statistical analysis revealed a significant difference in Mn levels among the sampling stations for *R. trilineata* ($p < 0.05$), whereas no significant differences were observed for *T. trichopterus* and *O. vittatus* ($p > 0.05$). Manganese is an essential trace element for both plants and animals, contributing to various physiological functions.

The concentrations of Cu, Fe and Mn in the analyzed fish species were within the acceptable limits established by the Food and Drug Administration (2007) [17] and the Food and Agriculture Organization (1983) [18], indicating no significant concern regarding heavy metal contamination in these species.

The findings of the present study were also compared with previous research. *M.A. Ashraf et al.* [19] reported that the concentration of Cu in *Osteochilus vittatus* from Kuala Selangor ranged between 2–4ppm, which is significantly higher than the Cu concentration observed in the present study (0.82ppm). In contrast, a study conducted in the Perak River [20] recorded lower Cu (0.013ppm) and Fe (0.038ppm) concentrations in fish compared to the present study.

Furthermore, the concentrations of Cu, Fe and Mn in *O. vittatus* were compared with studies conducted in Bulgaria [21] and Brazil [22]. The present study indicated relatively lower concentrations of these heavy metals in fish compared to both studies. However, comprehensive data on metal concentrations were not available in all previous studies.

Variations in heavy metal accumulation among different fish species have been widely documented. According to [23], disparities in heavy metal concentrations may be attributed to differences in ecological requirements, metabolism, feeding patterns and behavioral traits of fish species. Additionally, [24] suggested that the size and age of fish may influence metal accumulation. Fish can accumulate heavy metals in their tissues, particularly in muscle, through bioaccumulation. This process results in the gradual increase of contaminants within aquatic organisms as they absorb metals from their environment. The variability in heavy metal contamination sources leads to differing bioaccumulation levels across fish species refering to Wang, 2016 [25].

Conclusion

In conclusion, the concentrations of heavy metals in water samples from the TRIBE area exhibited the following increasing order: Cu > Fe > Mn > Al across all sampling stations. Copper (Cu) was found to have the highest concentration in water (0.579ppm), while iron (Fe) had the lowest concentration (0.0019ppm). Most of the heavy metal concentrations in water were elevated at station S3 (Al, Cu, Fe and Mn). Station S1 was classified as class II, which is suitable for recreational activities, while stations S2 and S3 were categorized as class III. However, the heavy metal concentrations in water samples remained below the permissible limits set by standard guidelines, indicating that TRIBE UniSZA is not significantly impacted by pollution or anthropogenic activities. In terms of heavy metal accumulation in freshwater fish, the order of concentrations was as follows: Fe > Al > Mn > Cu. A comparison of heavy metal levels and the associated p-values revealed significant differences in Fe concentrations among fish species. Although Fe levels were within acceptable limits for Rasbora trilineata and Trichogaster trichopterus, they may pose a concern for Osteochilus vittatus. Manganese concentrations were within acceptable ranges for all species, as different species exhibit varying abilities to accumulate metals. Future studies should consider extending the sampling period to encompass different seasons and additional sampling stations. This would allow for a more thorough examination of temporal and spatial variations in heavy metal concentrations, providing a comprehensive understanding of metal distribution within the ecosystem.

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