RESEARCH ARTICLE

Heavy Metals in the Striped Snakehead Murrel *Channa striata* and Sediments of Lake Mainit, Philippines with Notes on Piscine Micronuclei Occurrence

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ABSTRACT

Lake Mainit is the deepest lake in the Philippines, with sporadic documentation of various types of aquatic pollution. This paper reports the heavy metal content in the muscles of the striped snakehead murell *Channa striata* and bottom sediments from five stations across Lake Mainit using quadrupole-inductively coupled plasma-mass spectrometry (Q-ICP-MS). The micronuclei (MN) formation in erythrocytes of *C. striata* was also assessed for potential genotoxicity. The relative order of the average concentrations of heavy metals in *C. striata* samples across all stations is Zn>Cu>Cr>Ni>As>Pb>Cd. As per international safety standards, fish muscle samples across all stations have exceeded the permissible limits of Cu, Ni, and Zn, which has implications for health risks in humans consuming this important fish commodity. The relative order of the concentrations of heavy metals in sediments is Cr>Ni>Cu>Zn>As>Pb>Cd, where Cr, Ni, Cu, Pb, and Zn exceeded safety standards. The MN assay revealed that 98% of the erythrocytes assessed (N=147,000 RBCs) have normal cell morphology. Of the 2% of erythrocytic nuclear alterations (ENAs), 55% are found to be fragmented-apoptotic cells, while 31% are elongated or reshaped. The MNs in *C. striata* are relatively minimal at 2%. While the MN assay implies that *C. striata* from Lake Mainit are not at risk for potential genotoxic injury, the present study calls for seasonal monitoring for heavy metals in sediments and other fishery resources in Lake Mainit.

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1. INTRODUCTION

The freshwater environment is a recipient of many contaminants, a large proportion of which could have genotoxic potential (Claxton et al. 1998). Most water contaminants come from anthropogenic sources such as sludge or municipal compost, pesticides, fertilizers, emissions from municipal waste, and residues from ore mines and smelting industries (Yang et al. 2020; Ahamad et al. 2019). As a multifunctional surface water resource, lake ecosystems are particularly vulnerable to heavy metal pollution (Rashed 2001).

Lake Mainit is regarded as the deepest (219.35 m) and the fourth largest (17,060 ha) lake in the Philippines, with 28 river tributaries and only one outlet river that flows into Butuan Bay (Biña-de Guzman et al. 2013). This highly productive and diverse wetland ecosystem supports a thriving freshwater fishery for the municipalities of Surigao del Norte and Agusan del Norte. However, in recent decades, environmental and human factors have threatened the biodiversity and productivity of the lake. The lake is constantly influenced by anthropogenic activities from its surroundings, such as mining, unsustainable fishing techniques, agrochemicals, and the expansion of human settlements, which may have aggravated the lake's current condition (Paylangco et al. 2020).

A massive 2015 fish kill in Lake Mainit, mainly of Tilapia (*Oreochromis* sp.) and Carp (*Cyprinus* *carpio*), was linked to low dissolved oxygen. However, concentrations of heavy metals in the water and fish were not further examined during the incident (Catoto 2015). The study of Ebol and colleagues in 2020 reported trace concentrations of Pb in the muscles of *Oreochromis niloticus, Glossogobius giuris, Channa striata*, and *Vivipara angularis*, but values were within safe ranges. Trace Pb concentrations were detected in sediments across all seven stations only during the SW monsoon, with concentrations exceeding standard limits (31-91 ppm). However, Pb was below detectable limits across the seven stations during the NE monsoon (Ebol et al. 2020).

Heavy metals are one of the leading causes of nuclear aberrations, and pesticides from the agricultural sector pose a similar threat to aquatic ecosystems. It enters the aquatic ecosystem via runoff from agricultural fields, further polluting the aquatic ecosystems (Yohannes et al. 2013). A micronucleus (MN) test is a toxicological screening method for possible genotoxic substances. The MN test is being investigated in fish species to standardize and enhance the measurement of genotoxicity in target tissues (Da Rocha et al. 2009). This assay is widely regarded as one of the most effective and accurate methods for detecting genotoxic agents (Emam et al. 2014). Fish are often employed as excellent model animals for genotoxicological investigations when contaminants accumulate in the food chain as they offer early indications of toxicant-induced environmental changes (Pawar 2012; Hallare et al. 2011).

The freshwater snakehead, *Channa striata*, is a highly invasive fish established in many freshwaters in the Philippines, Indonesia, Madagascar, Taiwan, and Japan (Guerrero 2014). Its carnivorous and predatory nature enables the fish to obtain high concentrations of toxins from the surrounding environment (Lee and Ng 1991; Phoonaploy et al. 2019). *Channa striata* is a sentinel species because it is not threatened by extinction, is ubiquitous, and has an extensive distribution area and a stable population (Mustafa et al. 2021). In addition, its hardy nature and tolerance to poor water conditions make it a suitable indicator species (Phoonaploy et al. 2019).

Exposure and accumulation of heavy metals by fish can result in tissue burdens that adversely affect the exposed organisms and humans—the typical last consumer in the food chain (IARC 1993). The MN is an appropriate tool to warn early about a genotoxic threat to fish and other aquatic organisms, their ecosystem, and, finally, to humans. As one of the abundant species in Lake Mainit, studies on the exposure and effects of contaminants on *C. striata* are of interest to consumers and could serve as a basis for environmental managers and local policy implementers to develop food safety advice and regulations regarding the consumption of aquatic biota from the lake (Agtong et al. 2022). Given the above, the present investigations were carried out to determine the possible heavy metal contamination in the lake sediment and the accumulation of heavy metals in the muscles of *C. striata*. Potential genotoxicity through piscine erythrocytic nuclear alterations and micronuclei formation was also investigated.

2. MATERIALS AND METHODS

2.1 Research duration and locale

The fish sampling and collection of sediments were performed in October 2021. Five stations within Lake Mainit were established, namely: S1- Tagbuyawan; S2- Magtiaco; S3- Jaliobong; S4- Dinarawan; and S5- Kalinawan (Figure 1). These stations are major areas linked with anthropogenic activities. Small-scale and large-scale gold mining and processing operations are located around stations Tagbuyawan and Magtiaco. Jaliobong is surrounded mainly by agricultural regions, including rice fields, cornfields, and grasslands. Near the Kalinawan station are a lake resort and a cluster of residential residences, while an indigenous Mamanwa community and a nearby hydropower facility are located near the Dinarawan station.

2.2 Collection of specimen

A local fisherman assisted in collecting live adult-sized *C. striata* individuals with a spear at midday (8:00-10:00 am). Ten *C. striata* individuals were collected from each of the five study stations (N=50). The length (cm) and weight (g) of the captured fish samples were immediately measured using a ruler and digital weighing scale, respectively, on-site before performing the micronuclei assay.

2.3 Piscine Micronuclei Assay

The Piscine Micronuclei (MN) assay is an in situ biological indicator of chemical contaminant exposure. Following Ali et al. (2009), blood samples (triplicate of 1 drop or approximately triplicates of 50 µL from each fish individual) were collected from the ten *C. striata* individuals from each study station through a caudal vein puncture technique using a heparinized syringe and smeared in triplicates on clean glass slides.

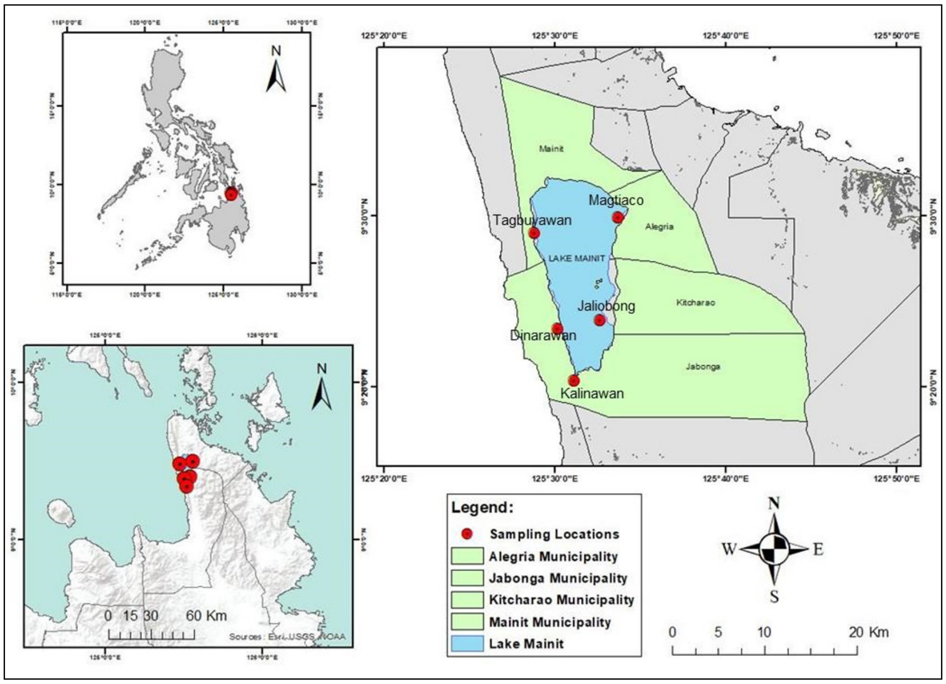

Figure 1. Stations established in Lake Mainit, Philippines.

Smears were fixed in absolute methanol for 25 min, followed by air drying the slides. Smears were stained with 10% Giemsa (Merck) for another 25 min. The slides were pre-coded in-house, and the entire analysis was carried out blindly; as such, the microscopist was unaware of the specific sampling stations of the slides scored to avoid unintentional bias in the investigation. Slides were examined to determine the frequency of cells with micronuclei occurrence and other patterns of erythrocytic nuclear abnormalities (ENAs) (Nirchio et al. 2019). Mature fish erythrocytes are typically oval and disk-shaped with a single compact nucleus (Da Rocha et al. 2009). A micronuclei (MN) formation is an additional erythrocyte formation not connected but with the same color and intensity as the central nucleus and has an area smaller than one-third of the main nucleus (Ali et al. 2009; Da Rocha et al. 2009; Cavas 2008). The ENAs are classified as aberrations other than MN formation in the smear. Morphologies of ENAs (e.g., binucleated, elongated/deshaped, notched, vacuolated, condensed, blebbed, fragmented-apoptotic, teardrop-shaped, segmented) followed descriptions from Da Silva-Souza and Fontanetti (2006), Cavas (2008), Carrasco et al. (1991), Nirchio et al. (2019), and Barsiene et al. (2014). In total, 1000 erythrocytes were accounted for each of the triplicate slides in a regular meander-like pattern noting MNs and ENAs at 1000× magnification under a light microscope (Motic®) equipped with a digital camera (Amscope®) (Nirchio et al. 2019; Ali et al. 2009). The frequency of MN and ENAs was calculated following Palacio-Betancur et al. (2009).

$$MN\ Frequency\ (\%) = \frac{Micronuclei\ \times\ 1000}{Total\ Cell\ Counted}$$

2.4 Sediment sampling and preparations for analyses

The grab sampling method was performed to collect bottom sediments where *C. striata* were also collected. Triplicate sediment samples (approximately 1 kg) in each station were collected using a modified scoop sampler (Ebol et al. 2020). Sample containers and sampling devices were thoroughly cleaned before use by soaking plastic containers and devices in 10% nitric acid (Simpson and Batley 2016). The preparation of sediment samples followed the method of Elvira et al. (2020). For 48 h, sediment samples were air-dried to remove moisture and oven-dried for 8 h at 600°C. Samples were sieved using sieves with the following mesh sizes: 2.00 mm, 850µm, 600µm, 425µm, 180 µm, 150µm, 106µm, 63µm, 38.5µm, and <38.5µm. The fractioned sediments were then weighed using a digital weighing scale. Furthermore, sieved sediment samples were used for particle size analysis to represent the comparative dimension of non-polycrystalline materials. Fractions from each sieve compartment were manually pulverized up to 5 µm following Elvira et al. (2020).

2.5 Processing of fish muscles

Packaging and preparing samples of *C. striata* muscle followed the method of Molina (2012). Fish muscles were removed on-site, placed on zip locks with labels, and brought to the laboratory for air and oven-drying. Preparation of the fish samples for digestion followed the method of Elvira et al. (2021). Samples were air-dried for 8 h and then oven-dried for another 16 h at 60°C. The samples were then pulverized using an agate mortar and pestle.

2.6 Digestion and analysis of muscles and bottom sediments for heavy metals

Digestion of *C. striata* muscles followed the method of Elvira et al. (2021). Briefly, 0.1 g of pulverized *C. striata* flesh samples were placed in a 15 mL Savillex Teflon vial with 7–8 mL of 7 M HNO3 and heated at 100°C for at least 24 h. The vial's lid was removed afterward to add 0.25 mL of 30% H_2O_2 and was heated at 100-120°C until it dried up. The sample was cooled and mixed with 0.714 mL of 7 M HNO_3 and 9.286 mL of Milli-Q water to produce a final solution of 10 mL 0.5 M HNO_3. The sample was covered and heated for 12 h at 115°C.

The digestion of sediment samples also followed the method of Elvira et al. (2020). Briefly, 0.1 g out of each sediment fraction (63µm) was collected and placed in a 15 mL Savillex Teflon vial with 5 mL of concentrated HF and 0.1 mL concentrated HNO_3. These were then covered with caps, heated for 3.5 h at 80°C, and dried gradually. Next, the samples were added with 7 mL of 7M HNO_3 before being heated at 100°C for 12 h. The caps were then removed, and the samples were continuously heated until dried. The samples were cooled down afterward before adding 7 mL of 6M HCl, and heated at 8°C for 12 h, covered with caps. The caps of the vials were removed and heated again at 120°C until dried. Approximately 7.14 mL of 7 M HNO_3 was added, and samples were heated at 100°C for 12 h to obtain the final solution. These were diluted 1,000 times and filtered with Advantec 5B 110 mm filter paper to make 100 mL of final sample solutions.

Quadrupole-inductively coupled plasma-mass spectrometry (Q-ICP-MS) (Agilent Technologies, 7700x) was used to determine arsenic, cadmium, chromium levels, copper, lead, nickel, and zinc in bottom sediments and fish. Calibration standards comprising 10 ng/g and 100 ng/g were produced in 0.5 M HNO_3 from a multi-element standard solution, ICP-MS-68A (High Purity Standards, USA). The calibration standards were measured after every ten unknown samples were analyzed.

2.7 Statistical Analysis

One-way ANOVA and Tukey's pairwise test set at $p \leq 0.05$ significance level were used to assess the statistical significance of the heavy metal concentration on fish and sediments across stations.

3. RESULTS

3.1 Piscine Micronuclei Assay in *C. striata*

Individual samples of *C. striata* (N=50; TL: 14.12 in ± 0.20; BW: 395.34 g ± 18.66) were collected across the five stations in Lake Mainit. Apart from the typical normal piscine RBC, 11 types of ENAs were documented (Figure 2; Table 1). From the total number of RBCs observed (N=150,000 cells), 98% of RBCs are of normal morphology (Figure 2A). Of the ENAs observed, 55% are fragmented-apoptotic cells, whereas 31% are elongated or deshaped. The MNs account for only 2% of the ENAs observed (Figure 2B).

3.2 Concentration of As, Cd, Cr, Cu, Pb, Ni, and Zn in muscles of *C. striata*

The relative order of the average concentrations of heavy metals in *C. striata* across all stations is Zn>Cu>Cr>Ni>As>Pb>Cd (Table 2). Fish samples from Magtiaco show significantly higher Cu (p=0.03) and Ni (p=0.02) than other stations. Samples across all stations have exceeded the permissible limits of Cu, Ni, and Zn as per international standards on fish muscles. Levels of Cr and Pb were below allowable limits, while Cd was not detected in this study.

3.3 Concentration of As, Cd, Cr, Cu, Pb, Ni, and Zn in sediments

The relative order of the average concentrations of heavy metals in sediments was in the order of Cr>Ni>Cu>Zn>As>Pb>Cd (Table 3). Sediment samples obtained from Tagbuyawan, Jaliobong, and Dinarawan exceeded Cr, Ni, and Cu permissible limits, while Zn, As, and Pb were below the standard limits. Nonetheless, Cr (p=0.00) and Ni (p=0.00) from Tagbuyawan sediments were significantly higher while the sediments from

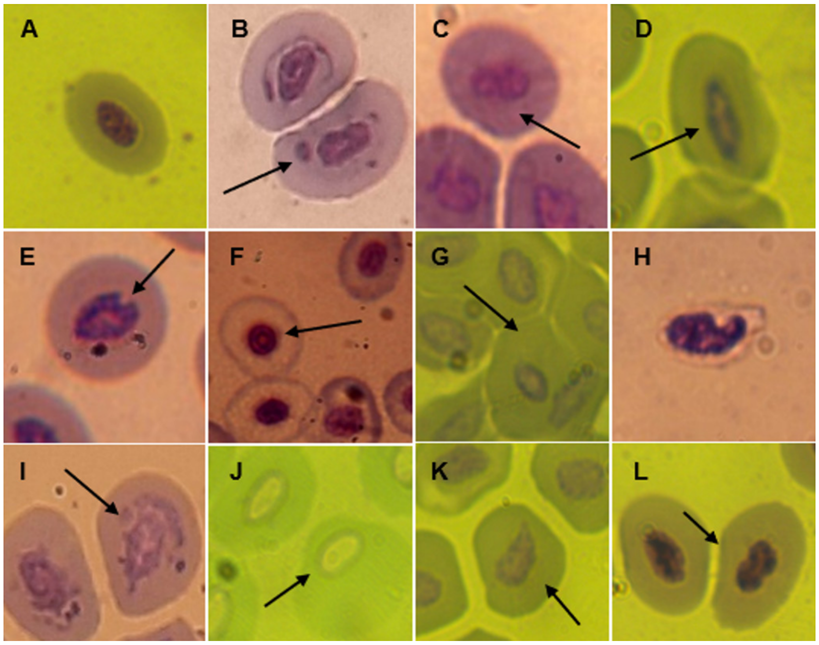

Figure 2. Representative images of various erythrocytic aberrations in *C. striata* collected from the stations surrounding Lake Mainit: (A) Normal cell; (B) Micronucleus (MN); (C) Binucleated cell (BN); (D) Elongated/Deshaped nucleus (EN); (E) Notched nucleus (NN); (F) Vacuolated nucleus (VN); (G) Condensed nucleus (CDN); (H) Blebbed nucleus (BLN); (I) Fragmented-apoptotic cell (FA); (J) Terminal-end nucleus (TN); (K) Teardrop-shaped nucleus (TDN); (L) Segmented nucleus (SN). N=150,000 RBCs at 1000 magnification.

Table 1. Description of normal and nuclear abnormalities in erythrocytes of *C. striata* from Lake Mainit.

Erythrocytic Nuclear Abnormalities	Description
Normal RBC	Nucleus is typically elliptical shape (Figure 2A).
Abnormal RBCs	
Micronuclei	MN's charomatin structure and staining intensity are comparable to that of the main nucleus. It has a diameter less than one-third that of the main nucleus, highly distinguishable from a binucleated cell (Figure 2B).
Binucleated cell	It is described as a cell with two nuclei (Figure 2C).
Elongated/deshaped	Its nucleus has a notably more unusual length than width, being longer and slender shaped (Figure 2D).
Notched	Nucleus has deeper invagination (Figure 2E).
Vacuolated	A "vacuole" in a nucleus appears as a well-defined hole with no visible substance. The vacuoles appeared to be separated by a membrane and has a consistent diameter throughout all observations (Figure 2F).
Condensed	Nuclei are smaller in size than the normal nuclei (Figure 2G).
Blebbed	Nucleus has a small invagination in its membrane (Figure 2H).
Fragmented-apoptotic cell	Early stages were distinguished by the presence of chromatin condensation inside the nucleus and intact cytoplasmic and nuclear boundaries, and late apoptotic cells display nuclear fragmentation into smaller nuclear bodies within an intact cytoplasm/cytoplasmic barrier (Figure 2I).
Teardrop-shaped	An erythrocyte's nucleus is deformed and tugged to a nipple shape at one end (Figure 2K).
Segmented	The nucleus usually contains lobes visible as nuclear division joined by thin strands of nuclear material, nearly observed as a binucleated cell (Figure 2L).

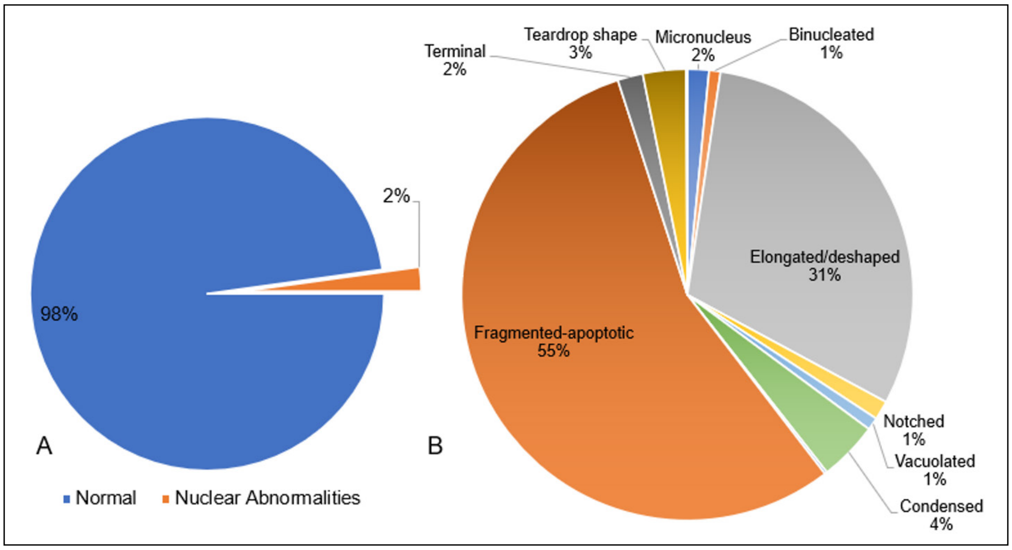

Figure 3. Percent (%) composition of RBCs in *C. striata* from Lake Mainit, Philippines. N=150,000 RBCs. A. % composition of RBCs with normal morphology and nuclear abnormalities. B. % composition of RBCs with nuclear abnormalities.

Table 2. Concentration of As, Cd, Cr, Cu, Pb, Ni, and Zn in collected *C. striata* from Lake Mainit, Philippines (mean±SEM).

Heavy Metals	Permissible Limit (ppm)	Concentration (ppm)					
		TAGBUYAWAN	MAGTIACO	JALIOBONG	DINARAWAN	MEAN	p-value
Cd	0.05[1]	n.d.	n.d.	n.d.	n.d.	n.d.	-
Cr	1[1]	0.62^{a} ± 0.12	0.53^{a} ± 0.17	0.09^{a} ± 0.01	0.78^{a} ± 0.21	0.31 ± 0.04	0.20
Cu	0.05[3]	0.95^{a} ± 0.03	1.65^{b} ± 0.21	0.73^{a} ± 0.05	0.74^{a} ± 0.03	1.02 ± 0. 04	0.03
Ni	0.05[1]	0.26^{a} ± 0.03	0.47^{c} ± 0.05	0.16^{b} ± 0.02	0.34^{a} ± 0.02	0.30 ± 0.01	0.02
Pb	0.5[1]	0.02^{a} ± 0.00	0.04^{a} ± 0.01	0.03^{a} ± 0.00	0.03^{a} ± 0.01	0.03 ± 0.00	0.48
Zn	30[3]	48.76^{a} ± 2.99	50.48^{a} ± 5.82	39.15^{a} ± 2.50	44.19^{a} ± 4.15	45.7 ± 0.74	0.68

n.d. = not detected; [1] FAO (1983); [2] FDA (1983), [3] WHO (1989); superscripts with the same letters indicate no significant difference ($p \geq 0.05$)

Table 3. Concentration of As, Cd, Cr, Cu, Pb, Ni, and Zn in <63 µm bottom sediments from Lake Mainit, Philippines (mean±SEM).

Heavy Metals	Permissible Limit (ppm)		Concentration (ppm)					MEAN	p-value
			TAGBUYAWAN	MAGTIACO	JALIOBONG	DINARAWAN	KALINAWAN		
As	10[1]	11[2]	4.6^{a} ± 0.6	3.95^{a} ± 0.2	6.01^{a} ± 0.8	5.4^{a} ± 0.5	4.40^{a} ± 0.05	4.8 ± 0.13	0.08636
Cd	0.99[1]	48[2]	n.d.	BDL	0.11^{a} ± 0	BDL	0.18^{a} ± 0.05	0.06 ± 0	-
Cr	43.4[1]	76[2]	1564.0^{b} ± 315.2	31.0^{a} ± 1.3	51.0^{a} ± 10.3	218.6^{a} ± 45.8	108.2^{a} ± 5.3	394.6 ± 60.4	0.0000704
Cu	31.6[1]	50[2]	43.1^{a} ± 3.6	155.8^{b} ± 11.9	156.1^{b} ± 19.5	91.9^{a} ± 5.9	207.2^{c} ± 11.6	130.8 ± 2.8	0.00001748
Pb	35.8[1]	0.65[2]	3.22^{a} ± 0.21	3.39^{a} ± 0.11	5.08^{a} ± 0.52	4.39^{a} ± 1.11	4.09^{a} ± 0.21	3.94 ± 0.18	0.2088
Ni	22.7[1]	24[2]	1520.5^{b} ± 277.3	12.3^{a} ± 0.1	30.3^{a} ± 11.3	156.2^{a} ± 43.1	53.6^{a} ± 3.1	354.4 ± 53.1	0.0000238
Zn	121[1]	14[2]	80.4^{a} ± 3.8	103.11^{a} ± 0.5	99.6^{a} ± 7.2	76.4^{a} ± 1.8	147.6^{b} ± 7.2	101.3 ± 1.4	0.00000923

n.d. = not detected; BDL = below detection limit; [1] USEPA; [2] Taiwan Environmental Protection Agency (TEPA)'s sediment quality guideline upper and lower limits (TEPA, 2010); superscripts with the same letters indicate no significant difference ($p \geq 0.05$)

Kalinawan were significantly higher for Cu (p=0.00) and Zn (p=0.00). Like the three stations, sediment samples from Kalinawan also exceeded the permissible limits of Cr, Ni, and Cu. However, concentrations of Zn also exceeded the set limits, while As and Pb were below the limits. Meanwhile, samples from Magtiaco have all the analyzed heavy metal concentrations below the standard limits except for Cu. Cd from all the stations was either minimal (0.1-0.2 ppm) or below detection limits. Compared to Ebol et al. (2020) study, Pb and Cd concentrations were lower in this study.

4. DISCUSSION

4.1 Piscine Micronuclei Assay in *C. striata*

Carrasco et al. (1991) first reported the occurrence of nuclear abnormalities (NA) in fish erythrocytes such as blebbed, lobed, notched nuclei, and binucleated cells. These nuclear abnormalities are considered biomarkers of genotoxicity (Ergene et al. 2007). The current study revealed a minimal appearance (2% of overall counted cells) of erythrocytic nuclear aberrations. Nonetheless, the observed nuclear abnormalities are thought to be indications of genotoxic damage and thus may support the scoring of micronuclei in standard genotoxicity assessments (Ergene et al. 2007).

Nuclear abnormalities in erythrocytes are caused by the toxicity of heavy metals from natural and anthropogenic activities (Cavas 2008). Heavy metals disrupt DNA strands and impair DNA synthesis, causing nuclear abnormalities (Tabrez et al. 2021). Heavy metals such as Cd, Pb, Cu, MeHg, Cr, Zn, and As were found to induce MNs, and other ENAs, namely nuclear bud, vacuolated nucleus, binucleated cell, condensed nucleus, kidney-shaped nucleus, segmented nucleus, lobed nucleus, notched nucleus, blebbed nucleus, and fragmented-apoptotic nucleus (Barsiene et al. 2006; Konen and Cavas 2008; Da Rocha et al. 2009; Monteiro et al. 2011). Likewise, agrochemicals such as pesticides, insecticides, herbicides, and fertilizers induce the appearance of ENAs in fish (Konen and Cavas 2008).

The % occurrence of MNs and ENAs is typically in minimal ranges ex-situ. The MN% was 0.19–0.22% in *C. striata*, and 0.15–0.25% in *C. punctatus* from heavily polluted sites in the Tamilnadu River, India. In the less polluted areas of the river, 0.05% and 0.07% MN in *C. striata* and *C. punctatus* were observed, respectively (Kuppu et al. 2018). Moreover, sublethal and prolonged exposure of toxicants in-situ produced a higher and more defined % MN as observed in *C. punctatus* exposed to chlorpyrifos (Ali et al. 2009). By comparison, exposure of *C. punctatus* to thermal power plant effluent with heavy metals lower than the concentrations in the current study was able to induce a high frequency of micronuclei (1133.3 %) and lobed nuclei (150 %) in-situ (Javed et al. 2015). Support in-situ studies relating nuclear and erythrocytic aberrations with sublethal heavy metal concentrations may be helpful to probe further if *C. striata* could qualify as a bioindicator or sentinel species using piscine MN assay under controlled conditions.

4.2 Concentration of As, Cd, Cr, Cu, Pb, Ni, and Zn in muscles of *C. striata*

Heavy metal bioaccumulation in fish can be influenced by several factors, including size, sex, reproductive cycle, dietary patterns, swimming behaviors, and living habitat (Zeitoun and Mehana 2014). Heavy metals are toxic and non-biodegradable and have bio-accumulative properties, making them an essential ecological and anthropogenic pollution indicator (Pragnya et al. 2021). The snakehead murrel is primarily carnivorous and prefers relatively deep (1-2 m), still waters (Lee and Ng 1991). Studies also indicate that heavy metals do not tend to accumulate at high muscle concentrations compared to the liver and gut. Nonetheless, our results show that *C. striata* collected from the Magtiaco station have higher Ni and Cu.

C. striata and *C. punctatus* in three major rivers in India have this order of accumulation of heavy metals: gills > liver > kidney > muscle. Further, Cd, Cu, and Pb showed higher bioaccumulation in the pre-monsoon samples, whereas As, Cr, and Zn exhibited higher bioaccumulation during the post-monsoon period (Kuppu et al. 2018). Similarly, the most significant quantities of Pb, Cr, Zn, As, Cu, and Ni were found in the gills. In contrast, allowable limits of these metals were found in the muscles and liver in *C. striata* and *Channa punctatus* from Kolleru Lake (Krishna et al. 2015). A 2020 survey in Lake Mainit has shown that muscle samples of all seven fish species, including *C. striata* assessed, were below detection limits (BDL) for tHg and Cd. Trace concentrations of Pb in the muscles were detected in *O. niloticus, G. giuris, C. striata*, and *Vivipara angularis* but values were within safe ranges (Ebol et al. 2020). The issue of Hg bioaccumulation and the safety of consumption for *C. striata* still needs to be resolved, as the current survey was unable to determine Hg concentration in the muscle samples.

4.3 Concentration of As, Cd, Cr, Cu, Pb, Ni, and Zn in sediments

The current survey utilized similar study stations to Ebol et al. (2020), except for collecting sediment samples in Mayag and Magpayang. The present research has provided an updated profile of the heavy metals in the sediments from the previous report. Ebol et al. (2020) reported Pb> Cd> tHg for two index seasons of collection, while the current study has shown the sediments to be Cr>Ni>Cu>Zn>As>Pb>Cd. The Tagbuyawan station, where mining activity is apparent, had the highest Cr and Ni levels, exceeding safety standards, and should be a cause for concern. Most recent surveys on the rice field soils adjacent to Lake Mainit also show high Ni (mean 61.3 ppm) and Cu (120.9 ppm). Regular heavy metal profiling of the lake bottom sediments, surrounding tributaries, and soil should be considered to understand better the seasonal dynamics and how it transcends HM accumulation in fish and humans.

5. CONCLUSION

This study reports the current heavy metal profile in the lake bottom sediments and *C. striata* in Lake Mainit while noting the occurrence of MNs and ENAs in the fish's erythrocytes to assess the possible genotoxic injury. The results reveal heavy metal pollution, as seen in concentrations of Cr, Ni, and Cu exceeding safe standards for sediments and fish muscles, which has important implications for health risks associated with consuming *C. striata* and other important freshwater commodities in the lake. Nonetheless, the piscine MN test appeared to lack sensitivity to the existence of the measured heavy metals since only minimal erythrocytic nuclear abnormalities were observed. Although the processes underlying NAs may not imply heavy metal contamination in the *C. striata* ex-situ, the observed nuclear aberrations may indicate genotoxic damage.

This study provides clear evidence that, indeed, pollution affects the economically essential resources in the lake. The entire ecosystem might be at risk if anthropogenic-enriched pollutants are not properly managed. Nonetheless, the result of this study is a sound basis for environmental managers and local policy implementers to develop food safety advice and regulations regarding the consumption of aquatic biota from the lake. Seasonal monitoring of heavy metals on sediments and aquatic fauna, as well as ecotoxicological and human health risk assessment, are recommended to provide a concrete narrative that biomagnification is happening.

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AUTHOR CONTRIBUTIONS

Laudiño FAR: Investigation, field data collection, Writing - original draft. **Agtong RJM**: Field data collection, sample preparation for HM analysis. **Elvira MV**: Conceptualized study design, Formal analysis, Validation, Writing – review and editing. **Fukuyama M:** HM analysis, Writing – review and editing. **Jumawan JC**: Conceptualized study design, Formal analysis, Writing – review and editing.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ETHICS STATEMENT

Channa striata is a shared resource for commercial and artisanal fishers and has an IUCN "Least Concern" status; hence, the protocol for the fish collection was not subjected to ethical clearance. Nonetheless, the number of individual *C. striata* collected for the study was minimized as far as practicably possible but sufficient to provide representative information. The authors also obtained consent from the local government units of the study stations to collect fish and sediment samples.

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