Environmental Research 224 (2023) 115517

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Toxic metal(loids) levels in the aquatic environment and nuclear alterations in fish in a tropical river impacted by gold mining

Leonomir Córdoba-Tovar [a,b], José Marrugo-Negrete [c,**], Pablo Andrés Ramos Barón [a], Clelia Rosa Calao-Ramos [d], Sergi Díez [e,*]

[a] *Pontificia Universidad Javeriana, Facultad de Estudios Ambientales y Rurales, Transversal 4#42-00, Bogotá, DC, Colombia*
[b] *Environmental Toxicology and Natural Resources Group, Universidad Tecnológica del Chocó, Quibdó, Choco, A.A. 292, Colombia*
[c] *Universidad de Córdoba, Cra 6 # 76 -103, Montería, 230002, Córdoba, Colombia*
[d] *Universidad de Córdoba, Facultad de Ciencias de la Salud, Programa de Bacteriología, Cra 6 # 76 - 103, Montería, 230002, Córdoba, Colombia*
[e] *Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research, IDAEA-CSIC, E-08034, Barcelona, Spain*

ARTICLE INFO

Keywords:
Atrato river
Sediment
Fish
Mercury
Micronucleus.

ABSTRACT

The Atrato River basin was protected by Colombian law due to anthropogenic impacts, mainly from illegal gold mining, which triggered a critical environmental health problem. In this study we quantified mercury (Hg), methylmercury (MeHg) and arsenic (As) concentrations in aquatic environmental matrices, and explored for the first-time nuclear degenerations in fish from the Atrato River. The median concentrations (μg/kg) for T-Hg, MeHg and As in fish were 195.0, 175.5, and 30.0; in sediments (μg/kg) 165.5, 13.8 and 3.1; and in water (ng/L), 154.7 for T-Hg and 2.1 for As. A 38% and 10% of the fish exceeded the WHO limit for the protection of populations at risk (200 μg Hg/kg) and for human consumption (500 μg Hg/kg); while As concentrations were below the international standard (1000 μg/kg) in all fish. The percentage of MeHg was 89.7% and the highest accumulation was observed in carnivorous fish (336.3 ± 245.6 μg/kg, $p < 0.05$) of high consumption, indicating risk to human health. In water, T-Hg concentrations exceeded the threshold effect value of 12 ng/L, whereas As concentrations were below the threshold of 10,000 ng/L, established by USEPA. On the contrary, 33% of the sediments exceeded the quality standard of 200 μg/kg for Hg. We found that *Prochilodus magdalenae* was the species with the highest susceptibility to nuclear alterations in its order, nuclear bud (CNB, 3.7 ± 5.4%), micronuclei (MN, 1.6 ± 2.5%) and binucleated cells (BC, 1.6 ± 2.3%). These results indicate that the species appears to be a good predictor of genotoxicity in the Atrato River. Fulton's condition factor (K) indicated that 31.7% of the fishes had poor growth condition, suggesting that the Atrato River basin needs to be monitored and restored in accordance with the agreements reached in the Minamata Convention on Mercury.

1. Introduction

Toxic metal pollution has been recognized as one of the global environmental challenges of greatest concern because it threatens human and wildlife (WHO, 2008). The presence of toxic metals, such as mercury (Hg) and metalloids such as arsenic (As), has increased considerably in aquatic systems around the world driven by anthropogenic activities such as agriculture and mining (UNEP, 2017; Wang et al., 2023). For Latin America and the Caribbean, it has been reported that small-scale artisanal mining was responsible for 29% of the Hg emissions released into the atmosphere in 2010. This percentage was equivalent to 208 tons of Hg out of the 727 tons globally, which represents a significant contamination of aquatic systems (Santana et al., 2014).

Globally, it is well documented that mining promotes the presence of Hg and As in the aquatic environment. Once these contaminants enter the water body they can precipitate to the bottom and accumulate in sediments and be present in the water column. In addition, they can increase their toxic potential as they react with other environmental constituents (Córdoba-Tovar et al., 2022; Wang et al., 2023).

When pollution occurs in aquatic environments, fish are one of the main receptors of contaminants, as well as good indicators of aquatic

---

* Corresponding author.
** Corresponding author.
E-mail addresses: jmarrugo@correo.unicordoba.edu.co (J. Marrugo-Negrete), sergi.diez@idaea.csic.es (S. Díez).

https://doi.org/10.1016/j.envres.2023.115517
Received 22 December 2022; Received in revised form 13 February 2023; Accepted 15 February 2023

pollution. This poses a risk in terms of human and environmental health, since fish is one of the most widely consumed sources of protein in most regions of the world (FAO, 2017; Garvey, 2019; Ré et al., 2021). For example, per capita fish consumption in 2019 amounted to 20.9 kg worldwide (Shahbandeh, 2019), whereas in Latin American, Colombia (7.16 kg) was the third country with the highest per capita consumption in 2017 after Peru (25.04 kg) and Brazil (9.09) (FAO, 2017).

In addition, it has been reported that high consumption of Hg-contaminated fish can increase nuclear damage in human populations (Galeano-Páez et al., 2021). Accumulation of toxic metal(loids) such as Hg and As in fish can reduce reproductive capacity, disrupt enzymatic processes, and substantially affect the immune system, increasing the risk of decline of mainly aquatic fauna (Hussain et al., 2018; Kumari et al., 2017). A worldwide review on the incidence of toxic metals in neotropicals freshwater fish indicated that Hg and As impose nuclear alterations ranging from the molecular level to behavioral modification in all life stages of the organism (Paschoalini and Bazzoli, 2021).

Nuclear alterations in aquatic organisms have been extensively evaluated by means of the micronucleus (MN) count or assay, a reliable and simple technique that provides valuable information on nuclear degenerations induced by different environmental stressors (Fenech et al., 1999; Obiakor et al., 2010, 2021; Vicari et al., 2012). The interpretation of genetic damage is the higher the frequency of the biomarker, the greater the DNA damage. In this sense, values higher than 0.3% indicate important effects in the organism (Carrola et al., 2014; Melo-Silva et al., 2018).

Numerous studies worldwide have reported on the effects imposed by toxic metal (loids) in aquatic environments mainly (Paschoalini and Bazzoli, 2021). However, there are knowledge gaps especially in areas prioritized as biodiversity hotspots such as the Atrato River basin (Abell et al., 2008). The Atrato watershed has been the main source of life for the riparian communities, but at the same time one of the most polluted in the region. For decades the watershed has received significant loads of toxic substances including Hg, which to a large extent have been products of economic activities including agriculture and gold mining (UNODC, 2016). In addition, many authors agree that human-induced socio-environmental impacts are often difficult to manage in developing countries, due to low investment in technologies and lack of political will to conserve biodiversity (Marrugo-Negrete et al., 2017; Marrugo-Negrete et al., 2018; Reid et al., 2020; UNEP, 2017; Vicari et al., 2012;Díez et al., 2011 ; Vörösmarty et al., 2010).

Under this scenario, in 2016 the Atrato River was protected with legal rights through sentence T-622-2016 issued by the Honorable Constitutional Court of Colombia (HCCC, 2016). The objective of this research was twofold: first, to quantify metal (loids) concentrations in water, sediments and fish, and second, to explore for the first time genetic degenerations in fish from the Atrato River.

2. Materials and methods

2.1. Study area

The Atrato basin is located in the Pacific region of Colombia in the department of Choco. According to the most recent census of the National Administrative Department of Statistics (DANE, for its acronym in Spanish), the overall population of the Atrato river basin is around 664,000 inhabitants, with most of the population concentrated in the Darien sub-region (DANE, 2018). The economy of the population of the Atrato basin is based on activities such as mining, agriculture, timber exploitation and fishing, where the latter registers an average consumption of 256 grams of fish per day (Salazar-Camacho et al., 2022). The work was carried out in five swamps in the Atrato River basin, hereafter referred to as stations.

The five stations were distributed between the middle and lower part of the Atrato River basin as shown in Fig. 1, and were selected and prioritized according to the use and importance for the communities. In the middle part the sampling stations were La Honda (CLH), Baudocito (CB) and La Sucia (CLS), and in the lower part Montaño (CM) and Los Medios (CLM). The basin has a length of 750 km, a surface area of 38,000 km^2 and an average annual flow of 4,137 m^3/s, and is the largest in the region. Forest and wetland ecosystems occupy 88%, agricultural land, pastures and urban settlements occupy 10%, and the water body occupies 2%. Annual rainfall in the basin ranges from 5,000 to 12,000 mm/year and the temperature is 26 °C (Palomino-Ángel et al., 2019; Velásquez and Poveda, 2019). Morphologically, the basin has an elongated synclinal shape due to a depression with sedimentary consequences. The geology and deposits of chemical elements are the result of weathering processes induced by high rainfall and temperature. The

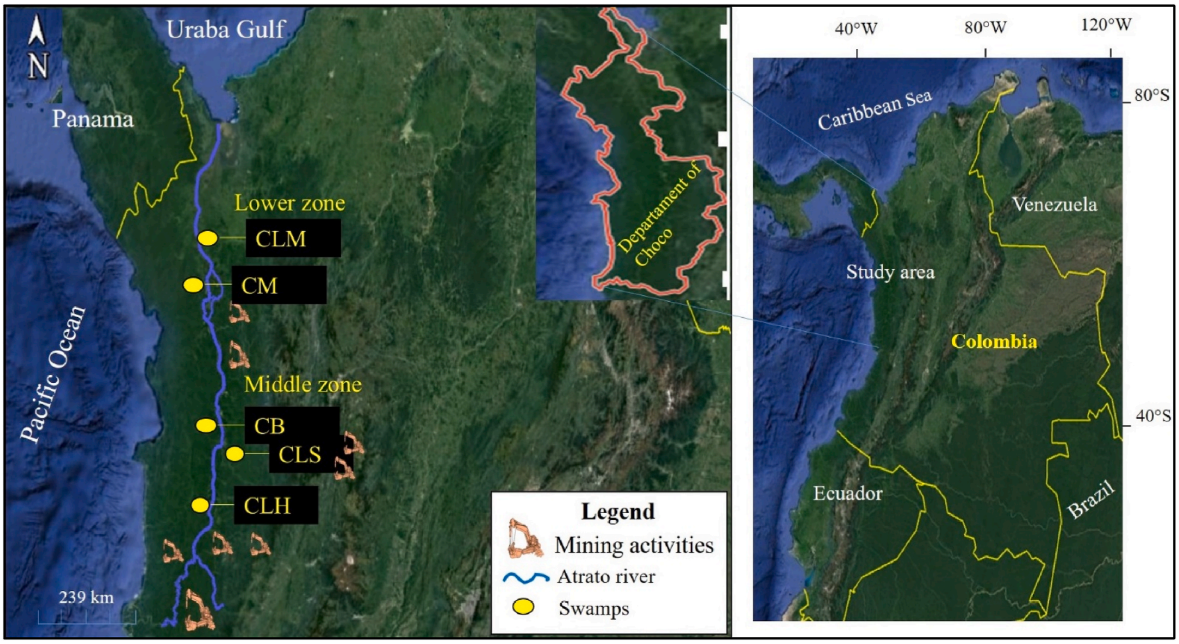

Fig. 1. Geographical location of the study area and sampling stations (swamps) in the Atrato River basin, Colombia. Los Medios (CLM), Montaño (CM), Baudocito (CB), La Sucia (CLS) and La Honda (CLH).

typical geological deposits are alluvium, beach sediments and terraces from the quaternary. The most relevant chemical elements in the basin are lead (Pb), copper (Cu), chromium (Cr), manganese (Mn), titanium (Ti), zinc (Zn), nickel (Ni) and cobalt (Co), especially in fine bottom sediments with important variations in the coastal zone and in the upper and middle parts of the basin (INGEOMINAS, 2003). In addition, the watershed receives significant loads of mining waste including toxic metals such as Hg (UNODC, 2016).

2.2. Collection of fish, water and sediment

A total of 205 fish were collected, grouped into 14 genus and 15 species. In each season, fish were collected randomly in the dry season. The number of fish per station were distributed as follows: CLH (n = 40), CB (n = 60), CLS (n = 20), CM (n = 49) and CLM (n = 36). All fish were caught with trammel nets directly by local fishermen. After capture, each specimen was locally identified and measured (total and mean length in cm) with a tape measure and weighed (in gram) with a digital weight (Pouilly et al., 2012). All fish collected were included in the study, as they were representative in the diet of the communities (Salazar-Camacho et al., 2022). With a scalpel from each fish, a portion of muscle of approximately 20–40 g was removed. All samples were stored in polyethylene bags and frozen until they were transferred to the laboratory. Taxonomic determination was done by means of illustrated keys, also taxa were corroborated in FishBase (2022).

Five water samples (250 mL) were collected at each station at a depth of not less than 20 cm depth collected with Van Dorm polycarbonate bottles. All samples were acidified with nitric acid and refrigerated until transferred to the laboratory (Gutiérrez-Mosquera et al., 2021; Marrugo-Negrete et al., 2015). Five sediment subsamples (0.5 kg) were collected with an Ekman dredge at each station at different cardinal points within a 2 m radius of the reference point. The samples were mixed until a single sample per station was obtained. The samples were stored in plastic bags, labeled and refrigerated until they were transferred to the laboratory (Marrugo-Negrete et al., 2015). Hg and As concentrations in the water samples correspond to unfiltered water.

2.3. Mercury and arsenic determinations

The quantification of total mercury (T-Hg) in fish and sediment samples was performed according to method 7473 indicated by the United States Environmental Protection Agency-USEPA (thermal decomposition, amalgamation and atomic absorption spectrometry). In water T-Hg concentrations were quantified by cold vapor atomic absorption spectrometry (CVAAS, Thermo Scientific iCETM 3500 - Waltham, MA, USA) after digestion with dilute KMnO4–K2S2O8 solutions for 2 h at 95 °C (USEPA, 1994). In fish, methylmercury (MeHg) concentrations were quantified following the methods EUR-25830 (Cordeiro et al., 2013) and EPA 7473 (USEPA, 1998). For MeHg measurement in sediment samples the methods EUR-25830 (Cordeiro et al., 2013), EPA 7473 (USEPA, 1998) and DMA 80 TriCell Milestone Inc., Italy (Maggi et al., 2009) were followed.

As concentrations were determined by hydride generation atomic absorption spectroscopy (HGAAS, SM3114B, Thermo Scientific iCETM 3500 - Waltham, MA, USA), with prior microwave-assisted acid digestion according to EPA method 3051 A for sediments (USEPA, 2007b), EPA 3015 A for waters (USEPA, 2007a) and AOAC method 999.11 for fish (AOAC, 2005). The precision/quality control of the method was performed using a certified National Research Council of Canada (NRCC) DORM-2 dogfish muscle standard. The percent recovery of T-Hg was 99.6 ± 0.2% (n = 3), and the detection limit (LOD) (3 μg/kg) was calculated as three times the standard deviation of a series of blank samples (n = 10). For MeHg the percentage recovery was 99.6 ± 3.7% (n = 3) with a LOD of 16 μg/kg. For quality control for As, calibration curves were constructed with R2>0.998, with a LOD of 60 μg/kg for fish, 1 ng/L for water and 49.66 μg/kg for sediment. Accuracy was evaluated using the reference material DORM-4 "Fish Protein Certified Reference Material for Trace Metals and other Constituents" recovering 6.81 ± 0.34 mg/kg of AsT (% recovery = 99.1%) with an RSD of 5.8%, being within the acceptance limits of the material (6.87 ± 0.44 mg/kg). Concentrations of T-Hg, MeHg and As were expressed in μg/kg ww of fish (ppb) and analyzed in duplicate (Marrugo-Negrete et al., 2015, 2020; Olivero-Verbel et al., 2016).

The distribution of Hg and As in environmental compartments in the Atrato river basin was evaluated through the partition or distribution coefficient (Kd) for Hg and As in sediments and water. This was expressed as the ratio between the concentrations of the metal in the solid phase and the aqueous phase. Its interpretation is based on the fact that low values of Kd indicate a greater degree of metal bias for the phase, while high values suggest a greater preference for the solid phase. In both matrices the units of measurement were converted to milligrams per liter (water) and milligrams per gram (sediment) to balance the coefficient ratio (Allison and Allison, 2005).

2.4. Micronucleus assay

Blood was smeared on microscope slides from each fish and three blood smears were performed per fish. The slides were cleaned with acetic acid and dried at room temperature for 3 h before spreading. The slides together with the blood sample were fixed with 99% methanol for 1 min and allowed to air dry for 10 min. After this time the slides were stained with Giemsa stain for 10 min. The slides were then washed with distilled water and left to dry at room temperature for 12 h (Hussain et al., 2018; Melo-Silva et al., 2018; Obiakor et al., 2014).

In the laboratory nuclear abnormality counting was performed on 2000 red blood cells using an optical microscope (Olympus BX43) and a 100x objective with 505–560 nm/objective filter under immersion oil. A total of 43 fish were examined randomly among the five stations, 33 in the middle part of the basin and 10 in the lower part for a total of 86,000 erythrocytes/fish (Calao-Ramos et al., 2021; Ré et al., 2021). Nuclear abnormalities including micronuclei (MN), binucleated cells (BC) and cells with nuclear bud (CNB) were used as evidence of cytotoxicity in fish *(*Obiakor et al., 2014*;* Ré et al., 2021*)*.

The frequency for each anomaly was evaluated by dividing the number of micronucleated erythrocytes by the total number of erythrocytes examined multiplied by 100 (Obiakor et al., 2021). The interpretation of genetic damage is that the higher the frequency of the biomarker, the greater the DNA damage. In this sense, values higher than 0.3% indicate important effects in the organism (*Carrola* et al., *2014*; *Melo-Silva* et al., *2018)*. Additionally, we used the weight (in g) and length (in cm) data of the fish under study to calculate the Fulton condition factor, which estimates fish welfare from the ratio *(K = 100 W/L3)*. A value of K ≥ 1 suggests a good growth condition or health of the organisms, while a K < 1 indicates a poor growth condition. This factor is a useful tool because it can easily be used as an indicator of the impact of pollution on aquatic systems *(Froese, 2006*; *Nash* et al.*, 2006)*.

However, it is worth mentioning that when evaluating the Fulton condition factor in Amazonian and/or tropical fish, the values can be affected by seasonal variations, partly because the food supply varies greatly with respect to the seasonal period *(Famoofo and Abdul, 2020*; *Froese, 2006)*.

2.5. Statistical analysis

The normality of the data was tested using normal probability plots. We assigned half of the value to the metal (loids) concentrations below the detection limit to properly perform the statistical analyses. A Shapiro-Wilk test was used for data sets <30 and a Kolmogorov-Smirnov test was used for data sets >30. A Mann-Whitney (U) test was used to determine differences between median T-Hg concentrations between stations according to their location in the Atrato River basin, i.e., lower and middle part. The same test was used to explore differences in T-Hg concentrations among fish when grouped according to their life form or feeding zone (e.g., pelagic, benthic and benthopelagic). Additionally, to reinforce any difference found, the effect of sample size was tested using the Hedges (g) hypothesis test (Hedges, 1981). A Kruskal-Wallis test was also used to determine differences in T-Hg concentrations among all stations, accompanied by a Dunn's post hoc test for multiple comparisons.

The degree of association between T-Hg and As concentrations and biometric variables (e.g., weight and length) of the fish was analyzed by Pearson's correlation; in this case, the data were transformed to logarithm with base 10. The concentrations of the metal (loids) in sediment and water were also logarithmically transformed to improve the graphical display only. A principal component analysis (PCA) was performed to explain possible associations between the concentrations of the contaminants evaluated in their aqueous and solid form with the concentrations found in the fish collected (Walters et al., 2017). A Spearman correlation between T-Hg and As concentrations and the percentage (%) of anomaly formation found in fish was used (Calao-Ramos et al., 2021). Statistical reporting included mean and median values, and for the level of statistical significance a probability of $p \leq 0.05$ was set. GraphPad Prisma (version 8.0) and Statgraphics (centurion XVI. I) were the statistical programs used for statistical analyses.

3. Results

3.1. Fish abundance and trophic grouping

Prochilodus magdalenae was the most abundant species within the total sample with 134 individuals (Table S1). The highest number of fish was collected at station CB (60 individuals, 29.3% of the total sample) and the lowest number at station CLS (20 individuals, 9.8% of the total sample).

According to FishBase (2022) of the 15 species, six were carnivorous, three detritivorous, two omnivorous, two omnivorous with a carnivorous tendency and two piscivorous. According to life form, the fish were grouped in percentage order into benthopelagic (89.8%), pelagic (9.8%) and benthic (0.4%), and two species were also found in the vulnerable category (Table S1). The trophic level of the fish ranged from 2.0 to 4.5. Carnivorous species were better represented within the overall sample (10 species; 66.7%) as opposed to non-carnivorous species (five species; 33.3%).

3.2. Total T-Hg and MeHg concentrations in fish muscle

The median T-Hg concentrations for all muscle samples analyzed, and the biometric characteristics of the fish are summarized in Table 1. Excluding species with $n = 1$, *Sternopygus aequilabiatus* (522.6 ± 139.6 μg/kg) and *Ageneiosus pardalis* (517.9 ± 129.6 μg/kg) were the two species with the highest T-Hg concentration, and *Leporinus muyscorum* (81.9 ± 67.7 μg/kg) and *Cyphocharax magdalenae* (78.3 ± 31.1 μg/kg) showed the lowest. T-Hg concentrations in fish collected at station CB (234.9 ± 151.4 μg/kg, $p < 0.05$) were statistically significantly different from those found at the other stations. We also observed that values in the middle part stations were statistically different and significant (209.0 ± 158.6 μg/kg, $p < 0.05$) vs. those in the lower part stations (Table S2). Of the total fish samples analyzed, 10% (21 individuals) exceeded the limit (Fig. 2) of allowable mercury concentrations for population protection set at 500 μg/kg (WHO, 1990) and 38% (78 individuals) exceeded the reference value for vulnerable or at-risk population set at 200 μg/kg (WHO, 2008). The remaining 52% (106

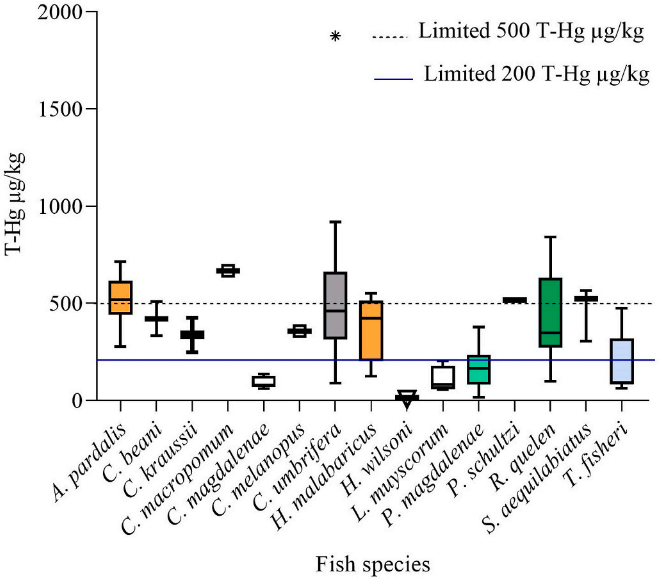

Fig. 2. T-Hg concentrations in fish collected in swamps of the Atrato River basin, Colombia. The dashed line refers to the thresholds recommended by FAO/WHO.

Table 1
Total mercury concentrations (T-Hg, median, standard deviation μg/kg, range) and biometric characteristics (TL = total length cm) and weight (W = weight in grams) of fish collected in swamps connected to the Atrato River basin, Colombia.

Scientific name	Common name	n	Diet*	Trophic level	T-Hg median	TL median ± SD Range	W median ± SD Range
Ctenolucius beani	Agujeta	2	C	4.0	420.7 ± 123.9 (333.1–508.3)	22.5 ± 2.1(21.0–24.0)	57.5 ± 3.5 (55.0–60.0)
Cathorops melanopus	Bagre blanco	1	C	4.3	357	43	643
Pseudopimelodus schultzi	Bagre sapo	2	C	3.7	516.5 ± 7.04 (511.5–521.5)	46.0 ± 4.2 (43.0–49.0)	614.0 ± 676.0 (136.0–1092)
Rhamdia quelen	Barbudo	7	OC	3.9	347.7 ± 249.3 (98.6–841.7)	32.0 ± 1.2(30.0–34.0)	251.0 ± 41.2 (203.0–295.0)
Sternopygus aequilabiatus	Beringo	3	C	3.2	522.6 ± 139.6 (305.4–566.1)	59.0 ± 5.7 (56.0–67.0)	355.0 ± 107.5 (250.0–465.0)
Prochilodus magdalenae	Bocachico	134	D	2.2	165.2 ± 88.9 (15.6–377.7)	27.0 ± 1.9 (22.0–34.0)	195.0 ± 52.9 (117.0–466.0)
Colossoma macropomum	Cachama	1	O	2.0	668.1	94	15.5
Trachelyopterus fisheri	Caga	9	P	3.5	90.3 ± 148.0 (62.9–473.7)	20.0 ± 2.9 (17.0–25.0)	66.0 ± 53.3 (36.0–180.0)
Leporinus muyscorum	Dentón	4	O	2.2	81.9 ± 67.7 (56.3–205.0)	34.0 ± 1.7 (33.0–37.0)	402.5 ± 91.1 (323.0–534.0)
Ageneiosus pardalis	Doncella	9	P	3.8	517.9 ± 129.6 (278.1–714.1)	23.0 ± 18.1 (20.0–67.0)	80.0 ± 737.8 (52.0–231)
Hemiancistrus wilsoni	Guacuco corroma	1	D	2.2	6.1	23	115
Cyphocharax magdalenae	Jojorro	7	D	2.0	78.3 ± 31.1 (60.1–136.6)	17.0 ± 0.5 (16.0–17.0)	60.0 ± 4.5 (54.0–68.0)
Caquetaia kraussii	Mojarra amarilla	2	OC	3.4	337.6 ± 126.4 (248.2–426.9)	21.5 ± 0.7 (21.0–22.0)	163.0 ± 39.6 (135.0–191.0)
Caquetaia umbrifera	Mojarra negra	12	C	3.8	459.6 ± 469.7 (88.8–1876)	23.0 ± 1.3 (25.0–20.0)	195.0 ± 34.1 (129.0–256.0)
Hoplias malabaricus	Quicharo	11	C	4.5	422.7 ± 151.4 (125.4–551.7)	34.4 ± 2.3 (30.0–37.0)	352.0 ± 151.4 (226.0–560.0)
Total		205			195.0 ± 181.0 (6.1–1876)	27.0 ± 8.4 (16–94)	195.0 ± 1084 (36–155)

Diet* = feeding habits: C = carnivore, O = omnivore, OC = omnivore with a tendency to carnivore, P = piscivore and D = detritivore. The information level trophic and diet was obtained from FishBase (https://fishbase.in/search.php).

individuals) were between 6.1 and 199.7 μg/kg.

We found a weak positive relationship between T-Hg concentrations, total length and standard length, and a null relationship between T-Hg concentrations and fish weight (Fig. S1). We found a positive and significant relationship between T-Hg concentrations and trophic level, indicating that trophic level was a relevant descriptor in explaining Hg variations in fish by 54% (Fig. S2). Results of MeHg in fish are presented in Table 2. The percentage of MeHg vs. T-Hg was 89.7%, furthermore the MeHg accumulation was higher and significant in carnivorous fish (336.3 ± 245.6 μg/kg, $p < 0.05$) compared to non-carnivorous fish (135.3 ± 81.4 μg/kg). In addition, T-Hg and MeHg concentrations showed a strong and statistically significant positive relationship ($r^2 = 0.996$, $p < 0.05$). Fish life form showed statistically significant differences in the level of T-Hg accumulation, i.e., scores in pelagics (median: 375.7; range: 62.9) were higher than those observed in benthopelagics (median: 184.8; range: 15.6), U = 111, p = 0.03, g: 0.94.

3.3. Total arsenic concentrations in fish muscle

The median As concentrations fish was 30.0 ± 36.8 μg/kg, being *C. beani* the species with the highest concentrations 167.5 ± 62.1 μg/kg. None of the fish samples (Table S3) analyzed reached concentrations equal to or above the maximum permitted value for human consumption established at 1000 μg/kg (FAO/WHO, 2002). Concentrations of As showed a positive relationship with the trophic level of the fish ($r^2 = 0.16$, $p < 0.05$). When we compared As concentrations between seasons, fish life form and biometric characteristics we did not observe significant differences ($p > 0.05$).

Table 2

Methylmercury concentrations (MeHg, median ± standard deviation, in μg/kg, range) and percentages (range and median ± standard deviation) in fish collected in swamps connected to the Atrato River basin, Colombia.

Scientific name	n	Trophic level	MeHg median ± SD	% MeHg	Range
Ctenolucius beani	2	4.0	384.3 ± 117.7	91.2 ± 1.1	90.4–92.0
Cathorops melanopus	1	4.3	275.7	77.2	–
Pseudopimelodus schultzi	2	3.7	465.5 ± 3.9	90.1 ± 0.4	89.8–90.5
Rhamdia quelen	7	3.9	287.1 ± 195.9	82.6 ± 7.7	73.2–94.1
Sternopygus aequilabiatus	3	3.2	470.3 ± 137.0	90.0 ± 5.5	84.2–90.5
Prochilodus magdalenae	134	2.2	142.7 ± 81.7	90.3 ± 6.1	75.4–102.4
Colossoma macropomum	1	2.0	547.6	82.0	–
Trachelyopterus fisheri	9	3.5	83.1 ± 137.3	86.1 ± 5.8	79.6–96.4
Leporinus muyscorum	4	2.2	76.6 ± 61.1	89.0 ± 7.0	79.3–96.6
Ageneiosus pardalis	9	3.8	475.0 ± 113.2	93.8 ± 5.8	81.2–97.8
Hemiancistrus wilsoni	1	2.2	5.3	86.1	–
Cyphocharax magdalenae	7	2.0	75.0 ± 26.5	88.2 ± 6.2	79.8–95.8
Caquetaia kraussii	12	3.4	320.9 ± 122.2	94.9 ± 0.6	95.4–95.5
Caquetaia umbrifera	2	3.8	379.6 ± 423.0	86.6 ± 6.0	75.0–92.1
Hoplias malabaricus	11	4.5	323.1 ± 132.1	91.3 ± 8.5	70.4–97.0
Total	205		175.5 ± 181.0	89.7 ± 6.3	70.4–102.4

3.4. Total Hg and as concentrations in water and sediments

At the stations in the middle part of the basin, the pH varied between 5.0-6.0 and 5.1–6.7 for the lower part, indicating moderately acidic waters. These values were within the normal ranges established in the Colombian environmental regulations for the preservation of aquatic biota (Decreto 3930, 2010). The behavior of T-Hg, MeHg and As concentrations in sediment and water samples collected at the five stations in the Atrato River is summarized in Fig. 3. Overall, metal (loids) concentrations in sediment ranged from 56.8 to 287.6 T-Hg μg/kg, 5.0–32.5 MeHg μg/kg and 2.6–3.5 As μg/kg with average concentrations of 165.5 ± 67.4 T-Hg μg/kg, 13.8 ± 9.1 MeHg μg/kg and 3.1 ± 0.2 As μg/kg. A 33.3% of the sediment samples (Fig. 3a) exceeded the sediment quality guideline of 200 μg/kg (USEPA, 2000). The MeHg and T-Hg ratio in sediment ranged from 3.6 to 13.67% with an average of 7.81%. In water, T-Hg concentrations ranged from 5 to 331 ng/L and 0.5–4.5 ng/L for As with an average of 154.7 ± 116.5 ng/L for T-Hg and 2.1 ± 1.1 ng/L for As (Fig. 3b). 60% of the water samples collected exceeded the 2000 ng/L threshold for T-Hg (USEPA, 1992), while As concentrations did not exceed the 10,000 ng/L threshold (WHO, 2001). However, median T-Hg concentrations in waters from the middle part of the basin were significantly different with respect to those found in the lower part (256.8 ±

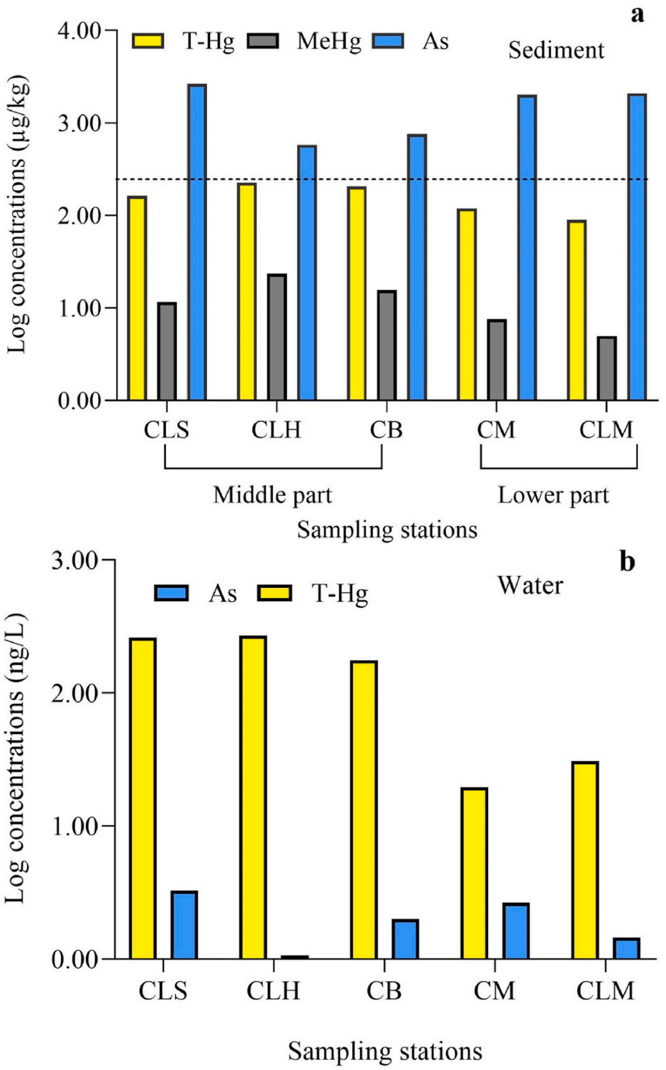

Fig. 3. Distribution of metal concentrations in water and sediment samples collected in different stations in the Atrato River basin, Colombia. The dashed line indicates the quality value of 200 μg/kg (USEPA, 2000).

52.0 ng/L, $p < 0.05$).

3.5. Principal component analysis

As shown in Fig. S4 the PCA explained 68.8% of the total variability of Hg and As concentrations found in fish. The PCA analysis also suggests that the concentrations of Hg and As in fish were largely associated with the concentrations of both contaminants in their sediment and aqueous phase.

3.6. Fulton's condition factor and nuclear abnormalities

Fulton's condition factor (K) for all fish ranged from 0.1 to 1.8 with an average of (1.0 ± 0.27). A 68.8% of the fish (140 individuals) exhibited a value of $K \geq 1$ indicating good health condition, whereas the remaining percentage 31.7% (65 individuals) suggest a poor health status. Stations CB (0.9 ± 0.1) and CLS (0.8 ± 0.1) contributed the highest number of fish with $K < 1$ values. Non-carnivorous species ($K = 1.1$, $p < 0.05$) showed a significantly better K factor than carnivorous species ($K = 0.9$). Additionally, the effect size test revealed that scores in non-carnivorous fish (median: 1.10; range: 0.8) were higher than those observed in carnivorous fish (median: 0.90; range: 0.1), $U = 315$, $p = 0.03$, g: 0.73, thus reinforcing the thesis that feeding habits have a strong effect on the health condition of fish when exposed to contaminated environments. CNB was the nuclear anomaly with greater frecuency with 79 observations (5.2 ± 5.7%) followed by MN (3.5 ± 2.4%), and BC (2.8 ± 2.3%) both with 43 observations among total 33 fishes sampled at the medium part of the river basin. *P. magdalenae* was the most sensitive species for all anomalies evaluated MN (1.6 ± 2.5%), CNB (3.7 ± 5.4%) and BC (1.6 ± 2.3%) in contrast to the rest of the fish analyzed (Table S4, Fig. 4). No alterations were evident among the total (10) fish examined in the lower part of the basin. However, it is striking that fish collected in the middle waters, where T-Hg levels in fish were higher, showed nuclear degeneration compared to fish collected in low waters. This suspicion suggests further research to help decipher more clearly the toxic agents responsible for nuclear degeneration in fish of the Atrato River basin. We know that in addition to Hg and As contamination, other xenobiotics (e.g., insecticides, pesticides) could be causing genetic damage in the aquatic biota of the Atrato basin, which means that for now, the contaminants responsible for the nuclear alterations in the fish of the basin remain undeciphered.

4. Discussion

4.1. Mercury species and as concentrations in fish

Among the fish collected ($n = 205$), a 10.2% exceeded the WHO maximum permissible value 500 μg/kg, (WHO, 1990), and it is not recommended for human consumption, whereas a 38.0% showed concentrations above 200 μg/kg (WHO, 2008), unsuitable for consumption by vulnerable populations, i.e., children under 15 years of age and pregnant women. The highest T-Hg concentrations were recorded in *S. aequilabiatus* (522.6 ± 139.6 μg/kg) and *A. pardalis* (517.9 ± 129.6 μg/kg). Additionally, carnivorous species including *C. umbrifera*, *H. malabaricus* and *C. kraussii* recorded higher concentrations with respect to non-carnivorous species (Table 1).

Previous studies in the Atrato River have shown that carnivorous species, including the species mentioned above, tend to have statistically significant T-Hg contents with respect to non-carnivorous species (Palacios-Torres et al., 2018; Salazar-Camacho et al., 2021). A recent work carried out in abandoned ponds at the same region, indicated similar trends (Gutiérrez-Mosquera et al., 2021).

T-Hg concentrations (375.7 μg/kg) in pelagic fish were different and significant ($p < 0.05$) when compared to those found in benthopelagic fish (184.8 μg/kg). These results suggested that part of the differences in Hg contents in fish are due to the lifestyle of the organisms. In that sense, Hg could be readily bioavailable to fish at the base of the benthic food chain (Lavoie et al., 2010). High Hg contents in pelagic organisms can be attributed to the interactions and complexity of benthic food webs (Arcagni et al., 2017; Muto et al., 2014). A summary of T-Hg concentrations in fish belonging to the same species analyzed in this study, from the same study area and other parts of the world is presented in Table 3.

T-Hg concentrations were higher in fish collected in the middle part (209.0 μg/kg, $p < 0.05$) as opposed to fish collected in the lower part of the Atrato River basin. In the study area, this behavior has been reported previously, with higher T-Hg concentrations in the middle part of the basin. These variations could be attributed to the association of Hg with particulate matter in the upper watershed, and also to the transport of contaminated sediment from upper watershed where mining is most intensively practiced (Palacios-Torres et al., 2018; Salazar-Camacho et al., 2021).

Hg and As concentrations in fish in the Atrato River appear to be related to availability in water and sediment. The PCA indicated that both environmental matrices may be acting as a potential source of contamination, suggesting that Hg and As may be readily available for uptake by fish. Both contaminants when present in the water column can be readily absorbed by the muscle tissues of organisms through the digestion process (Williams et al., 2010; Wang et al., 2023). In

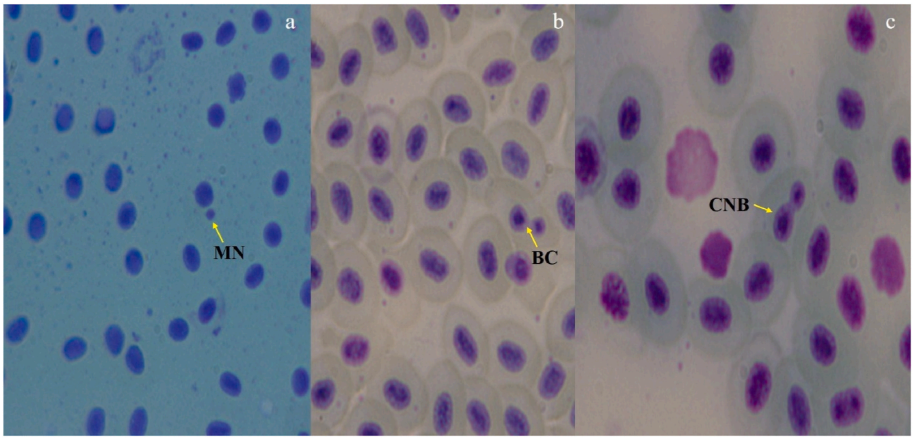

Fig. 4. Microscopic capture of micronucleus and other nuclear abnormalities in *P. magdalenae* species, a = micronucleated cells (MN), b = binucleated cells (BC) and c = cells with nuclear buds (CNB).

Table 3
Summary of T-Hg (μg/kg) concentrations in different fish of the same species examined in the same study area and in different parts of the world.

Scientific name	n	Environment	Departament	Country	T-Hg	Rerefence
Ageneiosus pardalis	57	Atrato River	Choco	Colombia	678.5	Salazar-Camacho et al. (2021)
	23	Atrato River	Choco	Colombia	950.0	Palacios-Torres et al. (2018)
Ctenolucius beani	28	Atrato River	Choco	Colombia	270.9	Salazar-Camacho et al. (2021)
Caquetaia kraussii	85	Atrato River	Choco	Colombia	218.0	Salazar-Camacho et al. (2021)
	44	Atrato River	Choco	Colombia	240.0	Palacios-Torres et al. (2018)
	2	Las Marías River	–	Venezuela	1020.0	Kwon et al., 2012
Cyphocharax magdalenae	18	Atrato River	Choco	Colombia	32.0	Salazar-Camacho et al. (2021)
	23	Atrato River	Choco	Colombia	60.0	Palacios-Torres et al. (2018)
Pseudopimelodus schultzi	22	Atrato River	Choco	Colombia	432.7	Salazar-Camacho et al. (2021)
Leporinus muyscorum	42	Atrato River	Choco	Colombia	116.7	Salazar-Camacho et al. (2021)
Prochilodus magdalenae	135	Atrato River	Choco	Colombia	93.1	Salazar-Camacho et al. (2021)
	26	Atrato River	Choco	Colombia	140.0	Palacios-Torres et al. (2018)
Rhamdia quelen	76	Atrato River	Choco	Colombia	145.8	Salazar-Camacho et al. (2021)
	2	Las Marías River	–	Venezuela	370,0	Kwon et al., 2012
Hoplias malabaricus	87	Atrato River	Choco	Colombia	401.4	Salazar-Camacho et al. (2021)
	46	Atrato River	Choco	Colombia	620.0	Palacios-Torres et al. (2018)
	5	Caquetá River	Amazonas	Colombia	720.0	Olivero-Verbel et al. (2016)
	11	Ayapel swamp	Cordoba	Colombia	840.0	Marrugo-Negrete et al. (2018)
	10	Urrá reservoir	Cordoba	Colombia	138.0	Marrugo-Negrete et al., 2015
	14	Itenez River	–	Bolivia	128.0	Pouilly et al. (2012)
	68	Madeira River	–	Brazil	240.5	Bastos et al., 2015
	51	Madeira River	–	Brazil	548.0	Bastos et al., 2007
	2	Río Las marías	–	Venezuela	1130.0	Kumari et al., 2017
Ageneiosus pardalis	9	Swamp, Atrato River	Choco	Colombia	517.9	This study
Ctenolucius beani	2	Swamp, Atrato River	Choco	Colombia	420.7	This study
Caquetaia kraussii	2	Swamp, Atrato River	Choco	Colombia	337.6	This study
Cyphocharax magdalenae	7	Swamp, Atrato River	Choco	Colombia	78.3	This study
Pseudopimelodus schultzi	2	Swamp, Atrato River	Choco	Colombia	516.5	This study
Leporinus muyscorum	4	Swamp, Atrato River	Choco	Colombia	81.9	This study
Prochilodus magdalenae	134	Swamp, Atrato River	Choco	Colombia	165.2	This study
Rhamdia quelen	7	Swamp, Atrato River	Choco	Colombia	347.7	This study
Hoplias malabaricus	11	Swamp, Atrato River	Choco	Colombia	422.7	This study

particular, in the aquatic environment inorganic As is absorbed by organisms, and then methylated to organic forms through different removal pathways that return arsenic to the water column, i.e., organic As concentrations may increase, due to the microbial activity present in the water (Azizur Rahman et al., 2012).

4.2. Mercury species and as concentrations in sediment and water

As participated with higher content in sediment compared to Hg, while Hg had higher content in water (154.7 T-Hg ng/L) with respect to As contents in the same matrix (2.1 ng/L). Although the concentrations for both elements were low in both matrices (water and sediment), the PCA results suggested that sediments could be one of the main sources of contamination in the ecosystems investigated. Additionally, the As contents in sediments were consistent with those reported in a previous study conducted in the same area (Palacios-Torres et al., 2020). The percentages of MeHg in sediments play a critical indicator role in predicting the bioavailability of Hg in aquatic media and in the water column, indicating that Hg would be readily bioavailable in plankton (Walters et al., 2017). In this same line, the fact that here the concentrations of Hg in water exceeded the threshold effect value, shows that contamination by this metal could impose toxic effects on aquatic biota (USEPA, 1992).

Here the MeHg and T-Hg ratio in sediment ranged from 3.6 to 13.7% with an average in concentrations of 7.82 μg/kg, which indicated that sediments could be acting as a relevant environmental descriptor of Hg concentrations in fish throughout the Atrato River basin (Gallego et al., 2018; Palacios-Torres et al., 2018; Salazar-Camacho et al., 2021). A recent study in the study area analyzed this metalloid in 1732 fish samples and none exceeded this value (Salazar-Camacho et al., 2022), even though, detection As in some aquatic matrices is a clear evidence of As pollution that can get worse over time (Palacios-Torres et al., 2020; Salazar-Camacho et al., 2022).

4.3. Distribution coefficients in sediment and water

The Kd determination values suggested that both contaminants Hg and As had a strong preference tendency for the solid phase despite low concentrations. In this study, the relationship between T-Hg and As concentrations in water and sediment for the five stations studied yielded a Kd of 67 and 79 for T-Hg and As, respectively (Fig. S3). We also observed a clear association between metal (loids) concentrations found in fish and aqueous and solid concentrations, indicating that both contaminants were readily available for uptake by fish. In addition, water and sediments could be two common sources of contamination (Melake et al., 2022; Walters et al., 2017).

In that sense, monitoring variations in concentrations as a function of annual season (i.e., winter and summer) could help to reduce uncertainties in estimating health risk (Allison and Allison, 2005), as the mobilization, distribution and accumulation of metals may be subject to physicochemical and biogeochemical conditions of the particular environment (Cui and Jing, 2019; Mukherjee et al., 2014; Paschoalini and Bazzoli, 2021). In general, Kd values for Hg when logarithmically transformed in aquatic environments (e.g., lakes, rivers and streams) show an average variation between 4.2 and 6.9 (suspended material/-water), 3.8–6.0 (sediment/water) and 2.2–5.8 (soil/water) globally (Allison and Allison, 2005). In addition, at low pH Hg tends to solubilize and adsorb as pH increases. Also, low pH can favor the formation of inorganic Hg at the solid-aqueous interface, i.e., sediment-water (Winfrey and Rudd, 1990). In this sense, Kd values can be affected by physicochemical factors including pH (Allison and Allison, 2005).

4.4. Growth condition and genotoxic effects in fish

The average value of the Fulton's condition factor (K) (1.0 ± 0.27) suggests that fish have good growth condition. However, values of $K \geq 1$ were observed in 68% of the fish. The remaining percentage (32%) showed a $K < 1$, indicating that the fish were not in good growth condition. Non carnivorous fish showed significantly ($K = 1.1$, $p < 0.05$) better growth condition compared to carnivorous fish ($K = 0.9$). The poor growth condition of fish suggests low fat deposition attributed to reduced food abundance and increased physiological demands for energy resources (De Jonge et al., 2015).

In this sense, the Fulton condition factor provides valuable information on fish welfare. It assumes that heavier individuals of a given length may exhibit better health conditions. However, species welfare can be affected by many factors including habitat, physicochemical conditions and seasonal variations at a given site (Froese, 2006; Oyebola et al., 2022). In addition, low K values could be an indicator of contamination, since it has been reported that in polluted places K values tend to decrease substantially. This could be attributed to the increased metabolic rate and increased fat metabolism by the organism, which is induced by the toxic action of a given pollutant (Javed and Usmani, 2019).

In relation to genetic damage, Hg for example, favors genetic instability by the loss of chromosomal fragments leading to the formation of micronuclei, which has been evidenced in humans (Galeano-Páez et al., 2021) and animals, mainly in fish (Hovhannisyan et al., 2017; Hussain et al., 2018). In that sense, changes and damage to genetic material in living organisms can be easily evidenced by counting cellular abnormalities including MN, CNB and BC. In addition, cellular alterations can be a clear evidence of organisms' response to the effects induced by toxic pollutants (Ahmad et al., 2008; Carrola et al., 2014; Hovhannisyan et al., 2017; Melo-Silva et al., 2018; Ré et al., 2021).

In our study, the micronucleus test helped in the determination of nuclear alterations in fish, despite not having established a robust design. Among the nuclear abnormalities, CNB had the highest occurrence in the total recount, followed by MN and BC both with the same number of observations. In general, all nuclear abnormalities reflected significant damage to the genetic code in the fish examined. Between the two species where genotoxic effects were clearly observed, *P. magdalenae* showed the highest MN formation (2.5%).

Similar results were reported in the Magdalena River basin, where *P. magdalenae* (0.23 ± 0.30%) showed higher MN formation compared to *Pimelodus blochii* (0.07 ± 0.11%). Moreover, these percentages were higher in sites highly contaminated with metals compared to other sites (Ortegon-Torres et al., 2014). According to worldwide research, percentages of MN, CNB and BC $\geq 0.3\%$ reflect significant genotoxic effects in organisms (Carrola et al., 2014; Melo-Silva et al., 2018). In *H. malabaricus* and *Prochilodus nigricans* in the Madeira River, Brazil, higher %MN (1.8%) have been reported in Hg-contaminated sites compared to non-contaminated sites (0.01%), which could be a clear evidence of the responsibility of impact of Hg in the genetic alteration of fish (Porto et al., 2005). However, genetic damage in fish induced by toxic metal (loids) such as Hg is related to the time of exposure and the level of bioaccumulation of the contaminant (García-Medina et al., 2017; Vicari et al., 2012).

Another thesis of particular importance in freshwater fish is the fact that genetic damage from metals, including Hg, has also been associated with toxicant-induced oxidative stress (García-Medina et al., 2017; Silvados et al., 2021). The occurrence of MN in fish of high importance in rural areas can be a useful tool in estimating and predicting the health risk associated with the consumption of fish in poor health conditions (Obiakor et al., 2014), because high average intakes of fish including *H. malabaricus* and *C. kraussii* have been shown to have a strong relationship with cytogenetic instability (e.g., %MN and %BC) in human populations (Galeano-Páez et al., 2021). Under this scenario, the riparian population adjacent to the study area could exhibit genotoxic effects, since the aforementioned species and others such as *P. magdalenae*, *A. pardalis*, *R. quelen* and *P. schultzi* are highly consumed in the region (Salazar-Camacho et al., 2022).

5. Conclusions

T-Hg, MeHg and As contents were analyzed in different environmental matrices at different stations in the Atrato River basin, and we found that T-Hg concentrations in fish, especially carnivorous species exceeded the reference values established by world health authorities, while As concentrations were below the threshold for fish. When we grouped the stations according to their location in the basin, T-Hg concentrations were statistically different in the middle part compared to the stations in the lower part of the Atrato River basin. The MeHg content found in fish suggests that the most toxic Hg species has a wide participation in the Atrato River basin increasing the risk to human health and aquatic fauna. Although there was no evidence of a relationship between T-Hg and As concentrations and genotoxic effects in fish, the micronucleus test revealed high percentages in the three categories of nuclear anomalies evaluated, indicating that fish show significant genotoxic damage due to environmental contamination. In addition, the species *P. magdalenae* appears to be an excellent sentinel to indicate environmental genotoxicity in the Atrato River basin. To our knowledge, this is the first research exploring genotoxic effects in aquatic environments in the region. These results, in particular, are important because they encourage the scientific community in the region to develop future research that will be more concerned with deciphering the toxic agents responsible for genetic degeneration in the organisms of the Atrato basin, since for the moment they remain hidden.

Credit author statement

Leonomir Córdoba-Tovar: Writing - Original Draft, Investigation, Methodology; Jose Marrugo-Negrete: Conceptualization, Supervision, Project administration, Funding acquisition; Pablo Andrés Ramos Barón: Supervision; Clelia Rosa Calao-Ramos: Investigation, Methodology; Sergi Díez: Conceptualization, Formal analysis, Supervision; Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors thank the Ministry of Science, Technology and Innovation - (MinCiencias), Colombia, for funding this research in the framework of the project: Evaluation of the degree of contamination by mercury and other toxic substances and its impact on human health in the populations of the Atrato River basin, as a result of mining activities. RC Agreement No. 849–2018, Minciencias Code: 1112-949-66291. The authors would like to thank the University of Córdoba for funding the project FCB-01-19, and to the communities of the Atrato River, especially to the fishermen who took part in the field sampling activities. Leonomir Córdoba-Tovar wants to thank MinCiencias for the fellowship Beca Bicentenario. This work was partly funded by the CSIC through the project iCOOP2021-COOPA20490. The authors would also like to acknowledge the CYTED (Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo), for financing the MercuRed Network (420RT0007).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

org/10.1016/j.envres.2023.115517.

References

Abell, R., Thieme, M.L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., Mandrak, N., Balderas, S.C., Bussing, W., Stiassny, M.L.J., Skelton, P., Allen, G.R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., Petry, P., 2008. Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. Bioscience 58 (5), 403–414. https://doi.org/10.1641/B580507.

Ahmad, I., Maria, V.L., Oliveira, M., Serafim, A., Bebianno, M.J., Pacheco, M., Santos, M. A., 2008. DNA damage and lipid peroxidation vs. protection responses in the gill of Dicentrarchus labrax L. from a contaminated coastal lagoon (Ria de Aveiro, Portugal). Sci. Total Environ. 406 (1–2), 298–307. https://doi.org/10.1016/j.scitotenv.2008.06.027.

Allison, J., Allison, T., 2005. *Partition Coefficients for Metals in Surface Water, Soil, and Waste* (Issue January 2005). Environmental Protection Agency, Washington, DC.

AOAC. (2005). AOAC 999.11-2005, 2006. *Lead,Cadmium, Copper, Iron , and Zinc in Foo*. AOAC 999.11-2005. Lead,Cadmium,Copper,Iron,and Zinc in Foo, 2006. http://www.aoacofficialmethod.org/index.php?main_page=product_info&products_id=1000.

Arcagni, M., Rizzo, A., Juncos, R., Pavlin, M., Campbell, L.M., Arribére, M.A., Horvat, M., Ribeiro Guevara, S., 2017. Mercury and selenium in the food web of lake nahuel huapi, patagonia, Argentina. Chemosphere 166, 163–173. https://doi.org/10.1016/j.chemosphere.2016.09.085.

Azizur Rahman, M., Hasegawa, H., Peter Lim, R., 2012. Bioaccumulation, biotransformation and trophic transfer of arsenic in the aquatic food chain. Environ. Res. 116, 118–135. https://doi.org/10.1016/j.envres.2012.03.014.

Calao-Ramos, C., Gaviria-Angulo, D., Marrugo-Negrete, J., Calderón-Rangel, A., Guzmán-Terán, C., Martínez-Bravo, C., Mattar, S., 2021. Bats are an excellent sentinel model for the detection of genotoxic agents. Study in a Colombian Caribbean region. Acta Trop. 224 (August) https://doi.org/10.1016/j.actatropica.2021.106141.

Carrola, J., Santos, N., Rocha, M.J., Fontainhas-Fernandes, A., Pardal, M.A., Monteiro, R. A.F., Rocha, E., 2014. Frequency of micronuclei and of other nuclear abnormalities in erythrocytes of the grey mullet from the Mondego, Douro and Ave estuaries-Portugal. Environ. Sci. Pollut. Control Ser. 21 (9), 6057–6068. https://doi.org/10.1007/s11356-014-2537-0.

Cordeiro, F., Gonçalves, S., Calderón, J., Robouch, P., Emteborg, H., Conneely, P., Tumba-Tshilumba, M.-F., Kortsen, B., Calle, B. de la, 2013. *IMEP-115: Determination of Methylmercury In Seafood* (Issue February). https://doi.org/10.2787/76278.

Córdoba-Tovar, L., Marrugo-Negrete, J., Barón, P.R., Díez, S., 2022. Drivers of Biomagnification of Hg, as and Se in Aquatic Food Webs: A Review. Environmental Research. https://doi.org/10.1016/j.envres.2021.112226, 204(October 2021.

Cui, J., Jing, C., 2019. A review of arsenic interfacial geochemistry in groundwater and the role of organic matter. Ecotoxicol. Environ. Saf. 183 (May), 109550 https://doi.org/10.1016/j.ecoenv.2019.109550.

DANE, 2018. Resultados Censo Nacional de Población y Vivienda Riosucio, Quibdó, Chocó. https://www.dane.gov.co/files/censo2018/informacion-tecnica/presentaciones-territorio/190806-CNPV-presentacion-Choco.pdf.

Decreto 3930, 2010. Por el cual se reglamenta parcialmente el Título I de la Ley 9 de 1979, así como el Capitulo II del Titulo VI-Parte III- Libro II del Decreto - Ley 2811 de 1974 en cuanto a usos del agua y residuos liquidos y se dictan otras disposiciones. https://www.minambiente.gov.co/wp-content/uploads/2022/02/decreto-3930-2010.pdf.

De Jonge, M., Belpaire, C., Van Thuyne, G., Breine, J., Bervoets, L., 2015. Temporal distribution of accumulated metal mixtures in two feral fish species and the relation with condition metrics and community structure. Environ. Pollut. (Amsterdam, Neth.) 197 (2015), 43–54. https://doi.org/10.1016/j.envpol.2014.11.024.

Díez, S., Esbrí, J.M., Tobias, A., Higueras, P., Martínez-Coronado, A. 2011. Determinants of exposure to mercury in hair from inhabitants of the largest mercury mine in the world.doi: 10.1016/j.chemosphere.2011.03.065.

Famoofo, O.O., Abdul, W.O., 2020. Biometry, condition factors and length-weight relationships of sixteen fish species in Iwopin fresh-water ecotype of Lekki Lagoon, Ogun State, Southwest Nigeria. Heliyon 6 (1), e02957. https://doi.org/10.1016/j.heliyon.2019.e02957.

FAO/WHO, 2002. Codex alimentarius—general standards for contaminants and toxinsin food. Schedule 1 maximum and guideline levels for contaminants and toxins in Food. Schedule 1 maximum and guideline levels for contaminants and toxins in food. Reference CX/FAC 02/16. Codex. https://www.fao.org/fao-who-codexalimentarius/thematic-areas/contaminants/en/?page=2&ipp=6&no_cache=1&tx_dynalist_pi1[par]=YToxOntzOjE6IkwiO3M6MToiMCI7fQ==.

FAO, 2017. Fish and seafood consumption per capita, 2017. https://ourworldindata.org/grapher/fish-and-seafood-consumption-per-capita?country=Eastern+Europe~MWI~ZAF~ATG.

Fenech, M., Holland, N., Chang, W.P., Zeiger, E., Bonassi, S., 1999. The HUman MicroNucleus Project - an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. Mutat. Res. Fund Mol. Mech. Mutagen 428 (1–2), 271–283. https://doi.org/10.1016/S1383-5742(99)00053-8.

FishBase, 2022. FisBase. FishBase-SeaLifeBase symposium: 30 Years of FishBase -15 Years of SeaLifeBase. http://www.fishbase.org/search.%0Aphp.

Froese, R., 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. J. Appl. Ichthyol. 22 (4), 241–253. https://doi.org/10.1111/j.1439-0426.2006.00805.x.

Galeano-Páez, C., Espitia-Pérez, P., Jimenez-Vidal, L., Pastor-Sierra, K., Salcedo-Arteaga, S., Hoyos-Giraldo, L.S., Gioda, A., Saint'Pierre, T.D., García, S.C., Brango, H., Espitia-Pérez, L., 2021. Dietary exposure to mercury and its relation to cytogenetic instability in populations from "La Mojana" region, northern Colombia. Chemosphere 265, 129066. https://doi.org/10.1016/j.chemosphere.2020.129066.

Gallego, S., Ramírez, C., López, B., Macías, S., Leal, J., Velásquez, C., 2018. Evaluation of mercury, lead, arsenic, and cadmium in some species of fish in the Atrato River delta, gulf of urabá, Colombian caribbean. Water Air Soil Pollut. 229 (8) https://doi.org/10.1007/s11270-018-3933-8.

García-Medina, S., Galar-Martínez, M., Gómez-Oliván, L.M., Ruiz-Lara, K., Islas-Flores, H., Gasca-Pérez, E., 2017. Relationship between genotoxicity and oxidative stress induced by mercury on common carp (Cyprinus carpio) tissues. Aquat. Toxicol. 192 (May), 207–215. https://doi.org/10.1016/j.aquatox.2017.09.019.

Garvey, M., 2019. Food pollution: a comprehensive review of chemical and biological sources of food contamination and impact on human health. Nutrire 44 (1). https://doi.org/10.1186/s41110-019-0096-3.

Gutiérrez-Mosquera, H., Marrugo-Negrete, J., Díez, S., Morales-Mira, G., Montoya-Jaramillo, L.J., Jonathan, M.P., 2021. Mercury distribution in different environmental matrices in aquatic systems of abandoned gold mines, Western Colombia: focus on human health. J. Hazard Mater. 403, 124080 https://doi.org/10.1016/j.jhazmat.2020.124080.

HCCC, 2016. Honorable Corte Constitucional de Colombia. Sentencia T-622 de 2016, Bogotá DC. http://www.corteconstitucional.gov.co/relatoria/2016/T-622-16.htm.

Hedges, L.V., 1981. Distribution theory for glass's estimator of effect size and related estimators. J. Educ. Stat. 6 (2), 107–128. https://doi.org/10.3102/10769986006002107.

Hovhannisyan, G., Harutyunyan, T., Liehr, T., 2017. Micronucleus fish. In: Thomas, L. (Ed.), Fluorescence in Situ Hybridization (FISH). Springer Protocols Handbooks, pp. 379–383. https://doi.org/10.1007/978-3-662-52959-1_40 (Issue September 2018.

Hussain, B., Sultana, T., Sultana, S., Shahreef, M., Ahmed, Z., Mahboob, S., 2018. Fish eco-genotoxicology : comet and micronucleus assay in fish erythrocytes as in situ biomarker of freshwater pollution. Saudi J. Biol. Sci. 25 (2), 393–398. https://doi.org/10.1016/j.sjbs.2017.11.048.

INGEOMINAS, 2003. Geología de las planchas,163 Nuquí, 164 Quibó, 184 Coquí y 184 Lloró en el departamento del Chocó, escala 1:100.000. https://catalogo.sgc.gov.co/cgi-bin/koha/opac-detail.pl?biblionumber=60965&query_desc=kw%2Cwrdl%3A%20163%20nuquí%2C%20164%20quibdó.

Javed, M., Usmani, N., 2019. An overview of the adverse effects of heavy metal contamination on fish health. Proc. Natl. Acad. Sci. India B Biol. Sci. 89 (2), 389–403. https://doi.org/10.1007/s40011-017-0875-7.

Kumari, B., Kumar, V., Sinha, A.K., Ahsan, J., Ghosh, A.K., Wang, H., DeBoeck, G., 2017. Toxicology of arsenic in fish and aquatic systems. Environ. Chem. Lett. 15 (1), 43–64. https://doi.org/10.1007/s10311-016-0588-9.

Kwon, S., McIntyre, P., Flecker, A.S., Campbell, L.M., 2012. Mercury biomagnification in thefood web of a neotropical stream. Sci. Total Environ. 92–97. https://doi.org/10.1016/j.scitotenv.2011.11.060.

Lavoie, R.A., Hebert, C.E., Rail, J.F., Braune, B.M., Yumvihoze, E., Hill, L.G., Lean, D.R. S., 2010. Trophic structure and mercury distribution in a Gulf of St. Lawrence (Canada) food web using stable isotope analysis. Sci. Total Environ. 408 (22), 5529–5539. https://doi.org/10.1016/j.scitotenv.2010.07.053.

Maggi, C., Berducci, M.T., Bianchi, J., Giani, M., Campanella, L., 2009. Methylmercury determination in marine sediment and organisms by Direct Mercury Analyser. Anal. Chim. Acta 641 (1–2), 32–36. https://doi.org/10.1016/j.aca.2009.03.033.

Marrugo-Negrete J., Pinedo-Hernández J., Díez S. 2015. Geochemistry of mercury in tropical swamps impacted by gold mining. doi: 10.1016/j.chemosphere.2015.03.012.

Marrugo-Negrete J., Enamorado-Montes G., Durango-Hernández J., Pinedo-Hernández J., Díez S., 2017. Removal of mercury from gold mine effluents using Limnocharis flava in constructed wetlands. doi:10.1016/j.chemosphere.2016.09.130.

Marrugo-Negrete, J., Ruiz-Guzmán, J., Ruiz-Fernandez, A., 2018. Biomagnification of Mercury in fish from two gold mining-impacted tropical marshes in Northern Colombia. Arch. Environ. Contam. Toxicol. 74 (1), 121–130. https://doi.org/10.1007/s00244-017-0459-9.

Marrugo-Negrete, J., Vargas-Licona, S., Ruiz-Guzmán, J.A., Marrugo-Madrid, S., Bravo, A.G., Díez, S., 2020. Human health risk of methylmercury from fish consumption at the largest floodplain in Colombia. Environ. Res. 182, 109050 https://doi.org/10.1016/j.envres.2019.109050.

Melake, B.A., Nkuba, B., Groffen, T., De Boeck, G., Bervoets, L., 2022. Distribution of metals in water, sediment and fish tissue. Consequences for human health risks due to fish consumption in Lake Hawassa, Ethiopia. Sci. Total Environ. 843 (October), 156968 https://doi.org/10.1016/j.scitotenv.2022.156968.

Melo-Silva, M., Oliveira, F.R., Rosa, J., Gemelli, E., Santos, L., Bueno-Krawczyk, A.C. de D., 2018. Comparison of nuclear Abnormalities in astyanax bifasciatus cuvier, 1819 (Teleostei: characidae) of two sections of rivers from the middle Iguaçu. Acta Sci. Biol Sci. 40 (1), 1–8. https://doi.org/10.4025/actascibiolsci.v40i1.40669.

Mukherjee, A., Verma, S., Gupta, S., Henke, K.R., Bhattacharya, P., 2014. Influence of tectonics, sedimentation and aqueous flow cycles on the origin of global groundwater arsenic: paradigms from three continents. J. Hydrol. 518 (PC), 284–299. https://doi.org/10.1016/j.jhydrol.2013.10.044.

Muto, E.Y., Soares, L.S.H., Sarkis, J.E.S., Hortellani, M.A., Petti, M.A.V., Corbisier, T.N., 2014. Biomagnification of mercury through the food web of the Santos continental shelf, subtropical Brazil. Mar. Ecol. Prog. Ser. 512 (October), 55–69. https://doi.org/10.3354/meps10892.

Nash, R.D.M., Valencia, A.H., Geffen, A.J., 2006. The origin of Fulton's condition factor - setting the record straight. Fisheries 31 (5), 236–238. https://folk.uib.no/nfiag/nfiag/reprints/NashETAL2006Fisheries.pdf.

Obiakor, M., Ezeonyejiaku, C., Ezenwelu, C., Ugochukwu, G., 2010. Aquatic genetic biomarkers of exposure and effect in Catfish (Clarias gariepinus, Burchell, 1822). Am.-Eurasian J. Toxicol. Sci. 2 (4), 196–202. https://www.academia.edu/26380220/Aquatic_Genetic_Biomarkers_of_Exposure_and_Effect_in_Catfish_Clarias_gariepinus_Burchell_1822_.

Obiakor, M., Okonkwo, J.C., Ezeonyejiaku, C.D., 2014. Genotoxicity of freshwater ecosystem shows DNA damage in preponderant fish as validated by in vivo micronucleus induction in gill and kidney erythrocytes. Mutat. Res., Genet. Toxicol. Environ. Mutagen. 775–776, 20–30. https://doi.org/10.1016/j.mrgentox.2014.09.010.

Obiakor, M., Tighe, M., Pereg, L., Maher, W., Taylor, A.M., Wilson, S.C., 2021. A pilot in vivo evaluation of Sb(III) and Sb(V) genotoxicity using comet assay and micronucleus test on the freshwater fish, silver perch Bidyanus bidyanus (Mitchell, 1838). Environmental Advances 5, 100109. https://doi.org/10.1016/j.envadv.2021.100109.

Olivero-Verbel, J., Carranza-Lopez, L., Caballero-Gallardo, K., Ripoll-Arboleda, A., Muñoz-Sosa, D., 2016. Human exposure and risk assessment associated with mercury pollution in the Caqueta River, Colombian Amazon. Environ. Sci. Pollut. Control Ser. 23 (20), 20761–20771. https://doi.org/10.1007/s11356-016-7255-3.

Ortegon-Torres, L., Henao-Murillo, B., Guio-Duque, A., Pelaez-Jaramillo, C., 2014. En peces de importancia comercial del río Magdalena , en el departamento del Tolima. Revista Tumbaga 53, 21–53.

Oyebola, O.O., Omitoyin, S.B., Hounhoedo, A.O.O., Agadjihouèdé, H., 2022. Length-weight relationship and condition factor revealed possibility of mix strains in Clarias gariepinus population of Oueme Valley, Benin Republic (West Africa). Total Environment Research Themes 3–4 (December), 100009. https://doi.org/10.1016/j.totert.2022.100009.

Palacios-Torres, Y., Caballero-Gallardo, K., Olivero-Verbel, J., 2018. Mercury pollution by gold mining in a global biodiversity hotspot, the Choco biogeographic region, Colombia. Chemosphere 193, 421–430. https://doi.org/10.1016/j.chemosphere.2017.10.160.

Palacios-Torres, Y., de la Rosa, J., Olivero-Verbel, J., 2020. Trace elements in sediments and fish from Atrato River: an ecosystem with legal rights impacted by gold mining at the Colombian Pacific. Environ. Pollut. (Amsterdam, Neth.) 256. https://doi.org/10.1016/j.envpol.2019.113290.

Palomino-Ángel, S., Anaya-Acevedo, J.A., Simard, M., Liao, T.H., Jaramillo, F., 2019. Analysis of floodplain dynamics in the Atrato River Colombia using SAR interferometry. Water 11 (5). https://doi.org/10.3390/w11050875.

Paschoalini, A.L., Bazzoli, N., 2021. Heavy metals affecting Neotropical freshwater fish: a review of the last 10 years of research. Aquat. Toxicol. 237 (July), 105906 https://doi.org/10.1016/j.aquatox.2021.105906.

Porto, J.I.R., Araujo, C.S.O., Feldberg, E., 2005. Mutagenic effects of mercury pollution as revealed by micronucleus test on three Amazonian fish species. Environ. Res. 97 (3), 287–292. https://doi.org/10.1016/j.envres.2004.04.006.

Pouilly, M., Pérez, T., Rejas, D., Guzman, F., Crespo, G., Duprey, J.L., Guimarães, J.R.D., 2012. Mercury bioaccumulation patterns in fish from the Iténez river basin, Bolivian Amazon. Ecotoxicol. Environ. Saf. 83, 8–15. https://doi.org/10.1016/j.ecoenv.2012.05.018.

Ré, A., Rocha, A.T., Campos, I., Marques, S.M., Keizer, J.J., Gonçalves, F.J.M., Pereira, J.L., Abrantes, N., 2021. Impacts of wildfires in aquatic organisms: biomarker responses and erythrocyte nuclear abnormalities in Gambusia holbrooki exposed in situ. Environ. Sci. Pollut. Control Ser. 28 (37), 51733–51744. https://doi.org/10.1007/s11356-021-14377-5.

Reid, A.J., Carlson, A.K., Hanna, D.E.L., Olden, J.D., Ormerod, S.J., Cooke, S.J., 2020. Conservation challenges to freshwater ecosystems. Encyclopedia of the World's Biomes 270–278. https://doi.org/10.1016/b978-0-12-409548-9.11937-2.

Salazar-Camacho, C., Salas-Moreno, M., Marrugo-Madrid, S., Paternina-Uribe, R., Marrugo-Negrete, J., Díez, S., 2022. A human health risk assessment of methylmercury , arsenic and metals in a tropical river basin impacted by gold mining in the Colombian Pacific region. Environ. Res. J. 212 (November 2021), 113120 https://doi.org/10.1016/j.envres.2022.113120.

Salazar-Camacho, C., Salas-Moreno, M., Paternina-Uribe, R., Marrugo-Negrete, J., Díez, S., 2021. Mercury species in fish from a tropical river highly impacted by gold mining at the Colombian Pacific region. Chemosphere 264, 2–10. https://doi.org/10.1016/j.chemosphere.2020.128478.

Santana, V., Medina, G., Torre, A., 2014. El Convenio de Minamata sobre el mercurio y su implementación en la región de América Latina y el Caribe. http://www.mercuryconvention.org/Portals/11/documents/publications/informe_Minamata_LAC_ES_FINAL.pdf.

Shahbandeh, M., 2019. Estimated fish consumption per capita worldwide in 2019, by region (in kilograms per capita). *Statista* (Issue 212). https://www.statista.com/statistics/1026312/global-fish-consumption-by-region/.

Silva, D., dos, S., Gonçalves, B., Rodrigues, C.C., Dias, F.C., Trigueiro, N.S. de S., Moreira, I.S., de Melo e Silva, D., Sabóia-Morais, S.M.T., Gomes, T., Rocha, T.L., 2021. A multibiomarker approach in the caged neotropical fish to assess the environment health in a river of central Brazilian Cerrado. Sci. Total Environ. 751, 141632 https://doi.org/10.1016/j.scitotenv.2020.141632.

UNEP, 2017. United Nations Environment Programme: towards a Pollution-free Planet Background Report. United Nations Environment Programme, Nairobi, Kenya. https://www.unep.org/resources/report/towards-pollution-free-planet-background-report.

UNODC, 2016. Explotación de oro de aluvión. Evidencias a partir de percepción remora. *Colombia*. https://repositoriobi.minenergia.gov.co/handle/123456789/2681.

USEPA, 1992. Water quality standards, establishment of numeric criteria for priority toxic pollutants. In: Federal Register, vol. 131, 246.

USEPA, 1994. Method 245.1: determination of mercury in water by cold vapor atomic absorption spectrometry. In: Environmental Sampling and Analytical Methods (ESAM) Program. https://www.epa.gov/esam/epa-method-2451-determination-mercury-water-cold-vapor-atomic-absorption-spectrometry.

USEPA, 1998. United state environmental protection agency. Method 7473 (SW-846). *Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absortion Spectrophotometry*. Methods. https://www.epa.gov/sites/production/files/2015-07/documents/epa-7473.pdf.

USEPA, 2000. Guidance for assessing chemical contaminant data for use in fish advisories. In: Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Vol. 1, Issue 4305. https://www.epa.gov/sites/default/files/2018-11/documents/guidance-assess-chemical-contaminant-vol1-third-edition.pdf.

USEPA, 2007a. Method 3015A: microwave assisted acid digestion of aqueous samples and extracts. In: *Environmental Sampling And Analytical Methods (ESAM) Program* (Issue February). http://www.epa.gov/osw/hazard/testmethods/sw846/online/3_series.htm.

USEPA, 2007b. *Method 3051A for Use of Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils* (Issue 235). http://digilib.unila.ac.id/11478/16/16. BAB II.pdf.

Velásquez, M., Poveda, G., 2019. Estimación del balance hídrico de la región Pacífica. Dyna 86 (208), 297–306. https://doi.org/10.15446/dyna.v86n208.73587.

Vicari, T., Ferraro, M.V.M., Ramsdorf, W.A., Mela, M., De Oliveira Ribeiro, C.A., Cestari, M.M., 2012. Genotoxic evaluation of different doses of methylmercury (CH3Hg+) in Hoplias malabaricus. Ecotoxicol. Environ. Saf. 82, 47–55. https://doi.org/10.1016/j.ecoenv.2012.05.007.

Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. Nature 467 (7315), 555–561. https://doi.org/10.1038/nature09440.

Walters, C., Couto, M., McClurg, N., Silwana, B., Somerset, V., 2017. Baseline monitoring of mercury levels in environmental matrices in the limpopo province. Water Air Soil Pollut. 228 (57) https://doi.org/10.1007/s11270-016-3230-3.

Wang, N., Ye, Z., Huang, L., Zhang, C., Guo, Y., Zhang, W., 2023. Arsenic occurrence and cycling in the aquatic environment: a comparison between freshwater and seawater. Water (Switzerland) 15 (1), 1–19. https://doi.org/10.3390/w15010147.

Who, 1990. Environmental health criteria 101. Methylmercury. World health organization. In: *Environmental Health Criteria* (Issue 101) sequence=1. https://apps.who.int/iris/bitstream/handle/10665/38082/9241571012_eng.pdf.

Who, 2001. Environmental Health Criteria 224 Arsenic and Arsenic Compunds, second ed. https://apps.who.int/iris/bitstream/handle/10665/42366/WHO_EHC_224.pdf?sequence=1.

Who, 2008. *Guidance For Identifying Populations at Risk from* (Issued by, Issue August). Issued by UNEP DTIE Chemicals Branch and WHO Department of Food Safety, Zoonoses and Foodborne Diseases https://wedocs.unep.org/bitstream/handle/20.500.11822/11786/IdentifyingPopnatRiskExposuretoMercury_2008Web.pdf?sequence¼1&isAllowed¼y.

Williams, C.R., Leaner, J.J., Nel, J.M., Somerset, V.S., 2010. Mercury concentrations in water resources potentially impacted by coal-fired power stations and artisanal gold mining in Mpumalanga, South Africa. Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering 45 (11), 1363–1373. https://doi.org/10.1080/10934529.2010.500901.

Winfrey, M.R., Rudd, J.W.M., 1990. Environmental factors affecting the formation of methylmercury in low pH lakes. Environ. Toxicol. Chem. 9 (7), 853–869. https://doi.org/10.1002/etc.5620090705.