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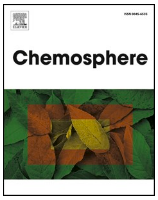

Metal bioaccumulation and genotoxicity in *Oreochromis niloticus* reared in farming pools influenced by mining activities in Napo, in the Ecuadorian Amazonia

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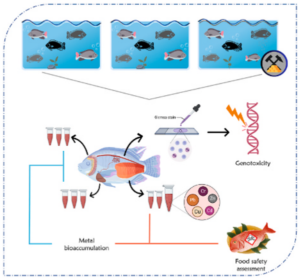
HIGHLIGHTS

- Cd, Cu, Cr, Pb, and Zn accumulation was detected in Tilapia reared in mining waste in non-mining areas in Ecuadorian Amazon.
- High genotoxicity was detected in tilapia reared in mining waste.
- Fish ingestion from all sampling sites leads to Pb and Cd ingestion 200-fold higher than maximum tolerable intake thresholds.
- Potential human health risks due to tilapia consumption are noted.
- Continuous monitoring is required to ensure food safety.

GRAPHICAL ABSTRACT



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ABSTRACT

Mining areas may suffer long-term metal contamination and represent harmful remnants of former mining activities. In the northern Amazon of Ecuador, former mining waste pits are used in *Oreochromis niloticus* (Nile tilapia) fish farming. Given the high consumption of this species by the local population, we aimed to estimate human consumption risks by determining Cd, Cu, Cr, Pb, and Zn tissue bioaccumulation (liver, gills, and muscle) and genotoxicity (micronucleus essay) in tilapia cultivated in one former mining waste pit (S3) and compare the findings to tilapias reared in two non-mining areas (S1 and S2); 15 fish total. Tissue metal content was not significantly higher in S3 than in non-mining areas. Cu and Cd were higher in the gills of tilapias from S1 compared to the other study sites. Higher Cd and Zn were detected in the liver of tilapias from S1 compared to the other sampling sites. Cu was higher in the liver of fish from S1 and S2, and Cr, in the gills of fish from S1. The highest frequency of nuclear abnormalities was observed in fish from S3, indicating chronic exposure to metals at

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this sampling site. The consumption of fish reared at the three sampling sites results in a 200-fold higher Pb and Cd ingestion than their maximum tolerable intake thresholds. Calculated estimated weekly intakes (EWI), hazard quotients (THQ), and Carcinogenic Slope Factors (CSF_{ing}) denote potential human health risks, indicating the need for continuous monitoring in this area to ensure food safety not only in areas affected by mining, but in general farms in the region.

1. Introduction

Metal pollution in aquatic ecosystems has become a major issue in the 21st century due to increasing anthropogenic activities (Kortei et al., 2020). In Ecuador, illegal mining activities have expanded in several Amazon basin regions, increasing the risks for environmental contamination (Capparelli et al., 2021). When abandoned, these mining areas are converted into food production areas, especially for fish farming, comprising a potential risk with regard to food safety and human health. The contamination level of fish reared in these former mining areas is, however, unknown. In the Napo province of Ecuador , for example, new mining concessions have been approved, and the current government has confirmed an increase in mining activities in the coming years (Galarza et al., 2021). Furthermore, several metals have been reported as routinely exceeding water and sediment quality standards in this province, with Pb and Zn above chronic contamination thresholds (Capparelli et al., 2021; Galarza et al., 2021).

Most metals are hazardous to aquatic biota and humans due to their environmental persistence and ability to bioaccumulate and, in certain cases, biomagnify, throughout aquatic trophic food webs (Cipriani-Avila et al., 2020). The dietary route is an important exposure pathway concerning metals, even in human populations not near conspicuous metal contamination areas, such as mining areas (Hauser-Davis et al., 2016). This is counterproductive to government and United Nations (UN) goals of establishing food safety-based programs, as nutrition is one of the main factors that directly affect human fertility and mortality rates (Figueroa and Rodríguez-García, 2002). Nevertheless, most governmental plans concerning this matter are focused on food amounts and availability, but not on its harmlessness (or lack thereof) (Friedrich, 2014).

In several Latin American countries, *Oreochromis niloticus* (Nile tilapia) farming constitutes an important economic activity and nutrient source (Carrera-Quintana et al., 2022). This species has been introduced as part of diet diversification strategies to increase the protein intake of human populations suffering restricted diets (Jácome et al., 2019). In Ecuador the introduction of this species to the Amazon region has been in course for several decades (Jácome et al., 2019). Tilapias are, in fact, now among the most consumed foodstuffs in both urban and rural Ecuadorian Amazonian areas and have been incorporated as part of local cultural food traditions as the main ingredient of the typical dish known as *Maito* (Jácome et al., 2019). Tilapia farming dependence is rising in rural areas due to population growth and human lifestyle changes from semi-nomadic to sedentary (Siren, 2015). In fact, *per capita* fish consumption among the indigenous Kichwa population of Napo was of 25.7 kg in 2011 (Siren, 2015), almost twice the annual consumption of 10.5 kg reported by Food and Agriculture Organization for Latin America and the Caribbean (FAO, 2022).

In the Ecuadorian Amazonia, abandoned mining ponds have been adapted to tilapia farming, comprising an alternative employed by mining companies to compensate landowners after the end of mining activities (Otchere et al., 2004). Potential metal contamination in this species, thus, becomes a significant human health concern, as tilapia from these areas are highly commercialized and consumed in the area (Otchere et al., 2004). Although some studies have indicated associations between metal-contaminated fish consumption and human risks in indigenous communities from Colombia, Peru, and Brazil (Carrera-Quintana et al., 2022), metal contamination data regarding fish reared in former mining ponds in Ecuador is scarce, mainly due to the absence of local food regulations and safety control. Therefore, this study aimed to assess metal contents in tilapia reared in former mining areas in the Ecuadorian Amazonia compared to non-mining areas and carry out human risk assessments concerning tilapia consumption. Furthermore, blood genotoxicity was also evaluated and associated with the detected metals, to evaluate the use of this contamination endpoint as a metal exposure biomarker in this species.

2. Material and methods

2.1. Sampling

Three popular tilapia marketing points were selected in the Tena and Arosemena Tola counties in the Napo province of Ecuador, home to several new and many abandoned gold mines (Capparelli et al., 2021; Roy et al., 2018). The sampling points comprise transition areas between the Andes and the Amazonian ecosystems (Fig. 1). Sampling Point 1 (S1, Fig. 1c), on the Tena-Talag road, in Sapo Rumi, and Sampling Point 2 (S2, Fig. 1d), on the Tena-Atacapi road, located within the buffer zone of the Colonso-Chalupas Biological Reserve, are areas built specifically for fish farming, while Sampling Point 3 (S3, Fig. 1e) is located on the Tena-Arosemena Tola road, a former mining area. Although not located in former mining areas, S1 and S2 cannot be classified as control sites *per se*, as they still undergo anthropogenic pressures (Capparelli et al., 2020).

Five tilapia specimens averaging 519.86 g, both males and females, were randomly obtained from each sampling point. The individuals were transported alive to the laboratory in coolers containing water from their pools. At the laboratory, fish were euthanized by spinal cord severing. Immediately, 0.50 ml of blood were taken from the caudal vein of each specimen with insulin syringes. A drop of each blood sample was placed on slide plates, and smears were prepared, in triplicate. The slides were then dried at room temperature and fixed with 90% ethanol, dried, and stored in a slidebox at room temperature until genotoxicity analysis (Nudi et al., 2010; Prieto et al., 2008).

Each fish was measured and weighed to obtain the Condition Factor (KF), used to determine fish physiological conditions (Campos et al., 2018), according to Equation (1).

$$KF = \frac{W}{L^3 \times 100} \tag{1}$$

where W is the body weight in grams and L is the body length in centimeters (Gusso-Choueri et al., 2016).

Three replicates of each tissue (gills, liver, and muscle) of the sampled 15 fish were obtained with samples. The 135 samples were then stored in sterile polypropylene tubes and frozen at −80 °C until metal content analysis, according to Gusso-Choueri et al. (2016).

2.2. Metal content determinations

The tissues samples were analyzed according to the Official Methods of Analysis of AOAC International (Association of Official Analytical Chemist, 2005). Each sample was defrosted at room temperature and homogenized with a mortar and pestle. Crucibles were then washed with 20% nitric acid, rinsed with deionized water, dried, and weighed. Homogenized samples were placed in the crucibles, weighed, and dried on a heat plate at ± 150 °C. The samples were then burned in a muffle starting at 200 °C, increasing 5 $°C \cdot h^{-1}$ until reaching 450 °C, maintained for 8 h. Samples were then allowed to cool at room temperature, and hydrated with 1–3 ml of distilled water. The water was evaporated in the hot plate at 150 °C, and the muffle procedure was repeated until white ashes were obtained. Then, 100 μl of a 10% HCl solution were added and mixed gently with the samples until all ashes became wet. The crucibles were subsequently covered with watch glasses and put on a hot plate inside a fume hood until complete acid evaporation. The residue was then diluted in 3.5 ml of HNO_3 65% w/w for 2 h and, finally, the samples were brought to volumetric capacity with distilled water in 25 ml plastic balloons.

Multi-elemental calibration curves were prepared using 1000 mg L^{-1} standard Cr, Zn, Pb, Cu, and Cd solutions (Merck) and the samples and blanks were analyzed employing a Thermo Scientific atomic absorption spectrophotometer with an air-acetylene flame burner and a graphite furnace. The Thermo SOLAAR software calculated Cr, Zn, Pb, Cu, and Cd concentrations. The instrument limits of detection and quantification (LOD and LOQ) were estimated as 6 standard deviations and 10 standard deviations of three blank measurements, respectively ($p < 0.05$). The instrumental LOD and LOQ are presented in Table 1 for each tilapia tissue.

Table 1

Instrumental Atomic Absorption Spectroscopy limits of detection and quantification (LOD and LOQ) expressed as μg·g-1 dry weight.

		Muscle		Liver		Gills	
		LOQ	LOD	LOQ	LOD	LOQ	LOD
Graphite furnace AAS	Cd	4.50E-06	2.14E-06	4.50E-06	2.14E-06	4.50E-06	2.14E-06
	Pb	1.02E-04	4.84E-05	1.02E-04	4.84E-05	–	–
	Cr	5.00E-06	2.37E-06	5.00E-06	2.37E-06	5.00E-06	2.37E-06
Flame AAS	Zn	3.00E-03	1.45E-03	3.00E-03	1.45E-03	3.00E-03	1.45E-03
	Pb	–	–	–	–	2.31E-02	1.09E-02
	Cu	7.80E-03	3.71E-03	7.80E-03	3.71E-03	7.80E-03	3.71E-03

2.3. Genotoxicity analyses

Plates were fixed with Carnoy's solution (methanol/acetic acid 3:1) for 15 min and stained with 2% Giemsa in phosphate buffer at pH 6.8 for 15 min. The slides were observed under a microscope at 100× magnification using immersion oil. The number of micronuclei (MN) and nuclear abnormalities (NA) were counted on a 1000-cell basis. Adapted from Nudi et al. (2010) and Prieto et al. (2008) (Nudi et al., 2010; Prieto et al., 2008).

2.4. Human health risks

Human health risks were calculated using the metal concentrations detected in tilapia muscle tissue. Estimated Weekly Intakes (EWI) were calculated for each metal to determine human consumption risks, using Equation (2), adapted from WHO (United Nations Environment Program & World Health Organization, 2008):

$$EWI = \frac{Metal\ concentration\ \left[\frac{\mu g}{g}\right] \times Weekly\ Fish\ Consumption\ [g]}{Population\ Body\ Weight\ [kg]} \tag{2}$$

The mean weight of the Ecuadorian human population in 2014 (Freire et al., 2014) was 67.9 kg, and the *per capita* Weekly Fish Consumption of 134 g·day^{-1} Siren (2015) as the average daily fish consumption of the Napo's Kichwa population, multiplied by 4, as people reported consuming fish 57% of weekdays (Siren, 2015). This value was employed considering that most of Napo's human population is indigenous, and the most significant ethnic nationality is the Kichwa (INEC, 2010). Data on fish consumption by the non-indigenous Ecuadorian Amazon population are not available.

Estimated Weekly Intake values were compared with the Provisional Tolerable Weekly Intake (PTWI) limits set by the joint FAO/WHO Expert Committee on Food Additive (JECFA) (Food And Drug Administration& World Health Organization, 2019; Köker, 2022; Miri et al., 2017; United Nations Environment Program & World Health Organization, 2008) to determine whether the consumption of tilapia in Napo represents a

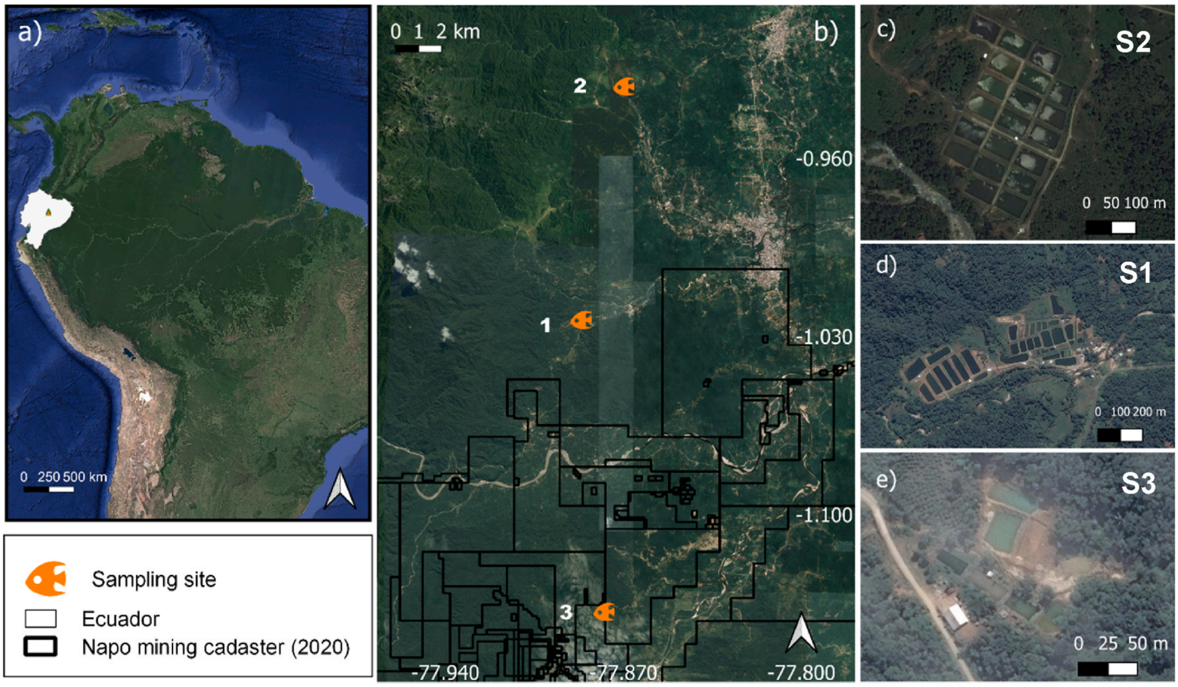

Fig. 1. a) Map indicating the study area located in the Napo province, Ecuador, on the Amazon River Basin; b) Fish symbols indicate the tilapia sampling points and black squared lines show the limits of gold mining concessions and fish farms. c, d and e) satellite imagery of each sampling site.

health risk.

The Target Hazard Quotient (THQ) set by the U.S. Environmental Protection Agency was also calculated to determine the risk of metal uptake from fish with non-carcinogenic effects, according to Equation (3) (Saha et al., 2016):

$$THQ = \frac{EDI}{RfD} \tag{3}$$

where EDI is the Estimated Daily Intake ($mg \cdot kg^{-1} bw \cdot day^{-1}$), calculated employing Equation (2) using daily (instead of weekly) Fish Consumption, RfD is the oral reference dose that represents the continuous risk to which the population is exposed to toxins without presenting noticeable deleterious effects, with values established as 0.001 mg kg^{-1}, 1,5 mg kg^{-1}, 0.04 mg kg^{-1} $mg \cdot kg^{-1}$, 0.004 and 0.3 mg kg^{-1} for Cd, Cr, Cu, Pb, and Zn, respectively (Saha et al., 2016). If the THQ is lower than 1, no health risks are noted, while values close to 1 indicate concerns.

The Cancer Risk assessment was obtained by the incremental probability of cancer development following a lifetime exposure (Equation (4)) (Saha et al., 2016).

$$CR_{ing} = EDI \times CSF_{ing}, \tag{4}$$

where the CSF_{ing} is the carcinogenic slope factor by ingestion set by FAO/WHO, USEPA and USDOE (Nkpaa et al., 2016), established as 0.38 $mg \cdot kg^{-1} bw \cdot day^{-1}$, and 0.0085 $mg \cdot kg^{-1} bw \cdot day^{-1}$ for Cd and Pb (Onyedikachi et al., 2018). Values between 10^{-6} and 10^{-4} are considered as predictive of cancer risk (Nkpaa et al., 2016).

2.5. *Statistical analyses*

All statistical analyses were conducted using the R software v4.02 (R Core Team, 2022). The averages of each replicate were obtained for each sampling point. Data normality was tested using the Shapiro-Wilk test. The Kruskall Wallis test was applied to compare tilapia condition factors between sampling points. The nuclear abnormalities observed at each location were compared using a one-way ANOVA test. A two-way ANOVA test was applied to assess the effect of metal concentrations among tilapia tissues and sampling points and to determine potential differences in the PTWI and EWI between sampling points.

3. Results and discussion

3.1. *Body condition factor (KF)*

We found that the mean tilapia KF at S3 was significantly higher than at S2 (Table 2). KF equation considers that individuals of similar length with greater body mass show better physiological condition (Campos et al., 2018). Nevertheless, KF may also be influenced by other factors such as water temperature and quality, fish tank population density, stress, environmental trophic status, and oxygen availability (Asmamaw et al., 2019; Saavedra, 2006). Also, one of the main factors that influence tilapia growth is feed amount and quality (Saavedra, 2006). Some aquaculture practices recommend a maximum feed intake to increase tilapia growth rates and reduce operational costs (Saavedra, 2006). In addition, metals such as Cu and Zn are commonly added to fish feed to accelerate tilapia growth and development (Wang et al., 2020). Such practices do not consider bioaccumulation processes for these metals in tissues, which may, therefore, represent a potential threat to both fish and consumers (Wang et al., 2020). Consequently, higher KF values do not necessarily represent better physiological fish conditions. Further studies are needed on fish feed composition at the three sampling sites to explain the differences in KF values among them.

Table 2
Tilapia condition factors per sampling point in the Napo province, Ecuadorian Amazonia, expressed as the means ± SD. Values in bold indicate significant differences between sampling points (Kruskal Wallis; df = 2; p < 0.05).

Sampling point	n	Body weight (g)	Total length (cm)	Condition Factor
S1	5	533.80 ± 74.08	24.80 ± 2.59	0.22 ± 0.02
S2	5	**461.60 ± 36.68**	23.80 ± 0.84	0.19 ± 0.01
S3	5	**564.20 ± 87.06**	24.60 ± 0.55	0.23 ± 0.03

3.2. *Metal content*

No significant effect of Cr concentration regarding sampling points was noted (Fig. 2). This agrees with other studies demonstrating little or no accumulation of this metal in fish (Nyeste et al., 2019; Saleh and El-Shahat, 2020), only occurring when excess Cr in liver and kidneys is observed (Li et al., 2022; Nyeste et al., 2019).

Cr concentration in the gills was significantly higher than in the liver. This would indicate that exposure to this metal has been recent (Campos et al., 2018) and that the liver is depurating Cr effectively (Rahman et al., 2018; Suchana et al., 2021). Here it is important to know that metal distribution in tissues is both species- and metal-specific (Parvin et al., 2019). No studies on metal affinity in tilapia have been found, but studies in other fish confirm that Cr has an affinity for gills (Hamid Dar et al., 2021). Other studies have also reported higher Cr accumulation in tilapia gills (Shah et al., 2020; Yılmaz et al., 2010). This altered concentration in gills could lead to altered fish respiration, decreasing oxygen concentrations and, potentially, death (Fawad et al., 2017).

Cu concentration was significantly higher in S1 fish than in S2 fish. The fact that fish at S3 exhibit high Cu bioaccumulation is consistent with the findings of Capparelli et al. (2021), who reported that Cu levels at this site are above national and international water quality standards (Capparelli et al., 2021). Also, the accumulation of this element in sites S1 and S3 is quite similar. Although S3 was initially considered the only site affected by mining, because it is located within the concessions, S1 was found to be affected by oil spills, artisanal and illegal gold mining, stone mining, poor waste management, and improper use of agricultural chemical inputs for more than ten years (GAD, 2019). The main Cu contamination sources in water and soil are mine tailings and wastewater sludges (Nordberg et al., 2022). This element is also present in mixtures used to disinfect fish tanks from various pathogenic microorganisms and ectoparasites (Gopi et al., 2019). In the study area, a copper sulfate, copper oxide, and calcium hydroxide mixture (termed Bordeaux broth) is used in agriculture as recommended by Ecuador's National Agricultural Research Institute (INIAP) (Vera et al., 2019; Villavicencio and Vásquez, 2008), probably accounting for the high Cu concentrations detected in tilapia tissues.

It was also found that Cu concentration in the liver was significantly higher than in muscle and gills. It has been reported that Cu mainly targets fish liver, gills, and kidneys (Hamid Dar et al., 2021). The liver usually accumulates high metal concentrations (Rahman et al., 2018), since it is the main detoxification organ in most vertebrates (Suchana et al., 2021; Viana et al., 2020). Therefore, the significant difference between the liver and gills would show that the fish are exposed to high concentrations of this metal and that liver is presenting problems detoxifying.

No significant differences in Pb bioaccumulation were found between sampling sites. Pb concentration in gills was found to be significantly higher than in liver and muscle. Pb has higher affinity for the kidneys, liver, and gills (Hamid Dar et al., 2021). It has been reported that when Pb accumulation is higher in gills, the route of entry was mostly through the water than by ingestion (Lee et al., 2019). Pb accumulated the most compared to the other metals of this study. This is consistent with other authors (Lee et al., 2019; Qiu et al., 2011), since Pb is one of the most accumulating toxic metals, as it easily binds to oxygen and sulfur atoms in proteins and forms stable complexes (Lee et al., 2019). Pb toxic effects in fish include physiological, behavioral, and biochemical damage and increased susceptibility to oxidative stress (Ihunwo, 2022 ; Lee et al., 2019).

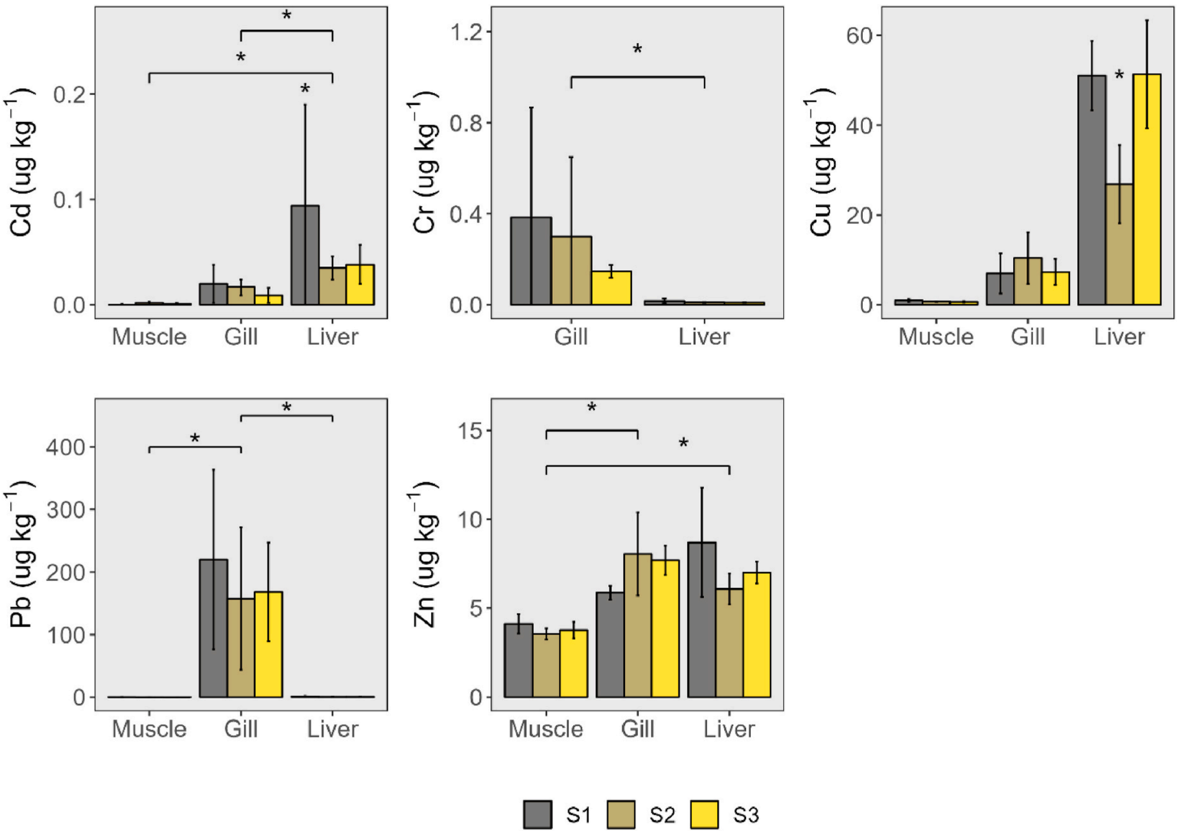

Fig. 2. Differences in tilapia metal bioaccumulation in muscle, gills, and liver between the three sampling sites in the Napo province, in the Ecuadorian Amazonia, expressed as means ± standard deviation. Bars above columns show statistically significant differences in metal concentration between tissues. (*) above columns show statistically significant differences in metal concentration in tissues between sites. (Two-way ANOVA; $p < 0.05$).

No significant differences were found between sampling points in Zn bioaccumulation. This metal accumulation was significantly lower in muscle than in liver and gills. Low Zn concentrations in fish muscle has been reported in other studies (Nyeste et al., 2019; Shahzad et al., 2018). However, Zn displays different bioaccumulation patterns, it decreases when body length increases (Hamid Dar et al., 2021). Also, the different bioaccumulation Zn pattern could be associated with feeding habits (Wang et al., 2020). At high concentrations, Zn could produce homeostasis issues, apoptosis, cytotoxicity, and cell necrosis (Briffa et al., 2020).

Bioaccumulation of Cd in fish from S1 is significantly higher than in S2 and S3. As explained above, S1 is an area that has been affected by various anthropogenic pressures. One of the main Cd contamination sources in fish tanks is agriculture drainage (Abougabal et al., 2020). This is consistent with reports of the presence of local agriculture, the main economic activity in the sampling area, focused on cacao, corn, banana, and cassava crops, among others (Gobierno Autónomo Descentralizado Parroquial Rural de Pano, 2019). Cd accumulation was found to be significantly higher in liver than in gills and muscle. This metal presents bigger affinity for the kidneys, liver, and gills (Hamid Dar et al., 2021). The liver is, in fact, the main Cd detoxification and bioaccumulation organ, and concentrations in this organ are usually proportional to those present in the environment (Rahman et al., 2018). In fish, Cd accumulation can result in oxidative stress, skeletal deformities, and organ damage (Abougabal et al., 2020).

The discussion whether the main sources of Cd contamination are natural or anthropogenic in the Amazon could be considered, since Amazonian soils can naturally contain high concentrations of this metal (Ribeiro et al., 2017; Siqueira et al., 2018). Cd concentrations above threshold levels have been reported in rivers in northern Ecuadorian Amazonia, suggesting anthropogenic contamination and/or lithogenic origins (Capparelli et al., 2021). Furthermore, if high Cd concentrations are to be biomagnified in fish edible tissues, farmculture activity in these areas must be evaluated to ensure the immediate safety of the local population.

Concerning sampling site, both Pb (221.06 μg·g-1) and Cd (0.11 μg·g-1) were detected at high concentrations in fish sampled from S1. Fish from S1 and S2 presented the highest metal levels. Site S1 is anthropogenically influenced, due to indiscriminate deforestation, sewage river discharges from nearby human settlements, garbage dumps, stone material extraction and, especially, livestock (Gobierno Autónomo Descentralizado Parroquial Rural de Pano, 2019). Capparelli et al. (2020) showed for the S2 moderate-to-high Cd, Pb, and Hg concentrations in water and Cd, Cr, Hg and Cu in sediments, and Galarza et al. (2021) showed poor water quality and little diversity of macroinvertebrates in this locality (Capparelli et al., 2020; Galarza et al., 2021). These authors indicate multiple sources of contamination for locations, including small-scale mining (illegal), sewage discharges, fish farming and non-functional landfills. In addition, these metals are also may be present in fish feed and some chemical products used in fishponds maintenance (Kalantzi et al., 2021), but may also be released from soil during pond building or naturally present in water (Nordberg et al., 2022). It is important to consider that the bioaccumulation also can be linked to many parameters such as species, kind of food, sex, life expectancy, exposure time, among others (Miri et al., 2017).

3.3. Human health risk assessment

The calculated Estimated Weekly Intake (EWI), Target Hazard Quotient (THQ) and Carcinogenic Risk (CR) for each sampling point and determined metal are displayed in Fig. 3.

A significant sampling point effect on the EWI was observed (Two-

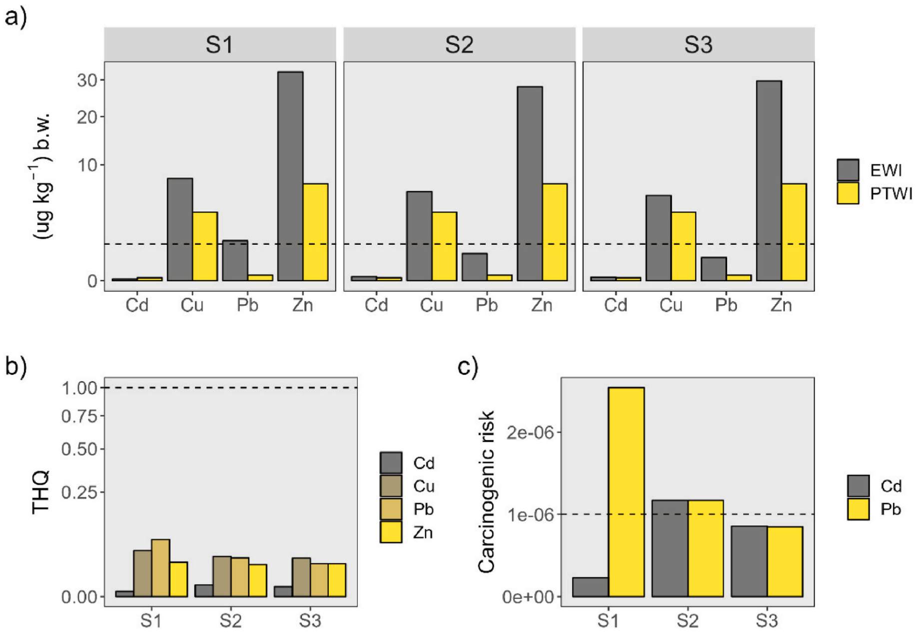

Fig. 3. a) Comparison between the Provisional Tolerable Weekly Intake (PTWI) and Estimated Weekly Intake (EWI) of the determined metals from the three different sampling sites (S). b) Target Hazard Quotient (THQ) for each metal and sampling site. c) Carcinogenic Risk by metal ingestion. The dashed line shows the minimum limit considered to predict carcinogenic risk. The y-axis has been square-root transformed to improve visualization in panels a and b.

way ANOVA; F (2,74) = 4.591; $p < 0.05$; η2 = 0.161), with higher EWI values calculated for S1 (M = 8.278) compared to both S2 (M = 6.885) and S3 (M = 7.096). A significant PTWI effect on EWI was also noted (two-way ANOVA; F (3,48) = 778.719; $p < 0.05$; η2 = 0.980).

Cu and Zn were above EWI at all sampling sites, while Pb was above the PTW at S1. Although Target Hazard Quotient (THQ) for all sites was below 1 (Fig. 3b), seemingly indicating no current risk for non-carcinogenic effects (Saha et al., 2016), the calculated Cancer Risk (Fig. 3c) were higher than the maximum threshold noted for Pb at S1 and for Cd and Pb at S2 regarding tilapia consumption from Napo. Nevertheless, since the EWI for Cu and Zn were above the permissible limits, and that Cr and Pb were higher in tilapia gills, indicating recent exposure, permanent monitoring should be conducted. However, health risks also depend on *per capita* fish consumption, body human weight, consumption frequency, and exposure time (Sharafi et al., 2019), which should also be considered, as well as potential additive or interactive effects of these contaminants on human health (Saha et al., 2016). In addition, Cancer Risk only considers one metal at a time and not their interactions.

Human health risks due to metal exposure are diverse. Exposure to Pb (Shah et al., 2020), Cu, and Cd, for example, all lead to nephrotoxicity (Nordberg et al., 2022). Lead, in particular, is dangerous even in low concentrations. This metal is responsible for up to 18% of annual deaths in the entire USA (Lanphear et al., 2018), and can result in severe chronic effects, as it is able to replace calcium in the body and lodge in the bones and blood system for years (Ishii et al., 2018). Furthermore, children with a history of Pb exposure exhibit inattention, psychological and school deficits, poor cognitive development, behavioral problems, physical violence, and crime rates when growing up (Khalid and Abdollahi, 2019; Needleman et al., 2002).

3.4. Genotoxicity analysis

Both MN and NA are evidence of damaged or broken chromosomes during cell division due to the presence of metallic pollutants in the mitotic apparatus (D'Costa et al., 2017; Gusso-Choueri et al., 2016) Furthermore, peripheral blood genotoxicity assessments are associated with metal body burdens and the destruction of the genetic material at a chromosomal level (Gusso-Choueri et al., 2016; Prieto et al., 2008). Micronuclei and Nuclear Abnormalities found in fish from this study are depicted in Fig. 4.

The frequencies of MN found in this study were 4.83, 3.33, and 14.19 per 1000 cells, while the frequency of nuclear abnormalities (NA) was 81.66, 45.27, and 183.71 per 1000 cells, for S1, S2, and S3, respectively (Fig. 5). A statistically significant effect of sampling location on MN (F (2,14) = 1.291; $p < 0.05$; $\eta^2 = 0.177$) was observed, where fish from S3 (M = 13.40) presented more MN than those from both S2 (M = 7.80) and S1 (M = 5.40). A significant effect of sampling location on NA was also observed (F (2,14) = 2.495; $p < 0.05$; $\eta^2 = 0.065$), where fish from S3 (M = 36.60) presented more NAs than those from both S2 (M = 12.60) and S1 (M = 17.36). This suggests higher genotoxicity in the former mining sampling point, corroborating the KF results of higher KF at S3. A previous study associated high fish weight with higher genotoxicity (Campos et al., 2021), potentially associated to metal exposure time, implying in higher genotoxicity levels (D'Costa et al., 2017). Genotoxicity may also be associated with the amount and type of food consumed by fish at S3, as fish feed contains both Cu and Zn (Wang et al., 2020), both of which were high in fish from S3. However, *O. niloticus* growth depends on factors such as development time, amount of feed, temperature, among others (Saavedra, 2006). However, due to S3 is an abandoned mining area, chronic exposure to metals is more likely to cause genotoxicity (Barbosa et al., 2010), thus, our findings seemingly indicate long-term pollutant exposure. The contaminated environments can cause inhibition of DNA repair processes as well, resulting in high levels of genotoxicity, but a negative correlation sometimes occurs, which may depend on the fish species, sex and age (D'Costa et al., 2017).

4. Conclusions

Fish from the mining area presented higher KF than the other sites, potentially due to feed contamination. The results of the genotoxic analysis are consistent with the hypothesis of higher genotoxicity in fish reared in the mining area. These results point out a state of deterioration of water quality at S3, caused by metal contamination and genotoxic activity. It is recommended to establish a monitoring program for the presence of genotoxic in fish grown in mining zones. The consumption of

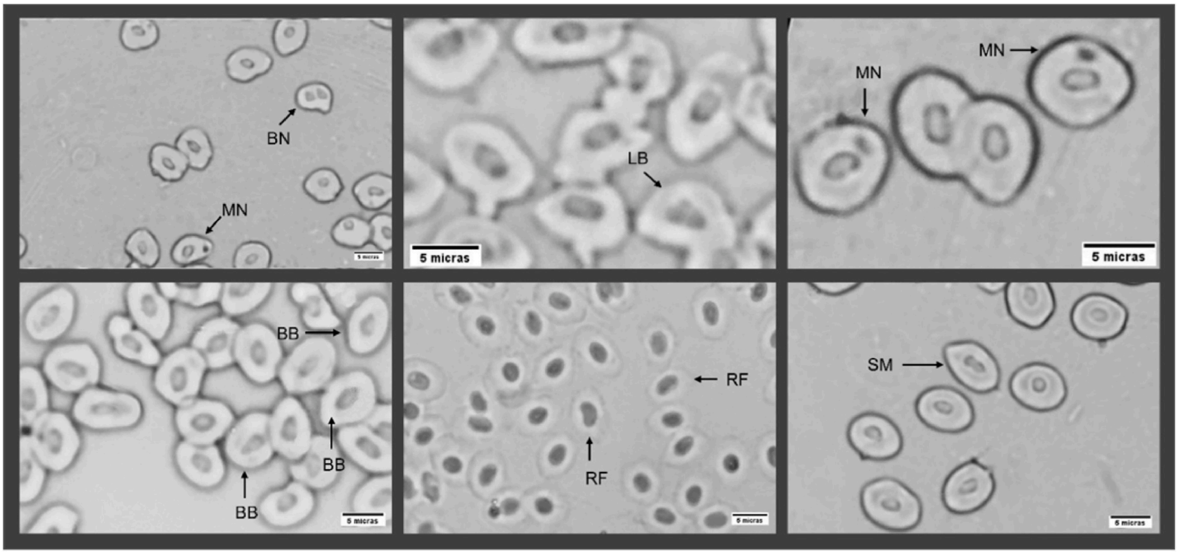

Fig. 4. Micronuclei (MN) and Nuclear Abnormalities (NA) registered in tilapia's peripheral blood from Napo. MN: Micronuclei, BN: Binucleated, RF: Reniform, LB: Lobed, SM: Segmented, BB: Blebbed.

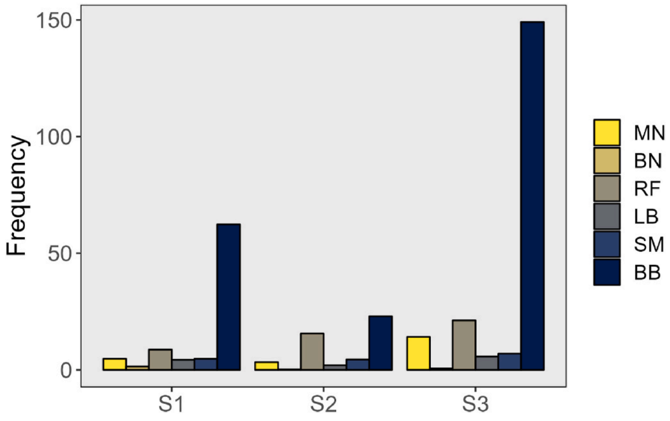

Fig. 5. Frequency of nuclear abnormalities in *Oreochromis niloticus* erythrocytes of. MN: Micronuclei, BN: Binucleated, RF: Reniform, LB: Lobed, SM: Segmented, BB: Blebbed. Frequency was calculated for 1000 cells.

farmed tilapia in the northern Ecuador Amazonia region for Cu and Zn were higher than established EWI limits at all sampling sites, while Pb was above the PTW at S1. Cancer Risk were noted for both Cd and Pb, even though the THQ were below 1. Thus, the indigenous Kichwa seem to be at risk from the consumption of tilapia reared in former mining areas.

Author contributions

Conceptualization: MVC, SVV, MC; Data curation: APG, MVC, MC, SVV, GMM,; Formal analysis; SVV, GMM, MC, APG; Funding acquisition: MVC, GMM, APG; Investigation: MVC, SVV; Methodology: SVV, MVC, GMM, APG; MC; Project administration: MVC; Validation: MVC, RAHD, GMM, MC, APG; Writing – original draft: SVV, MVC, MC, RAHD; Writing – review & editing: SVV, MVC, GMM, RAHD.

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.

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