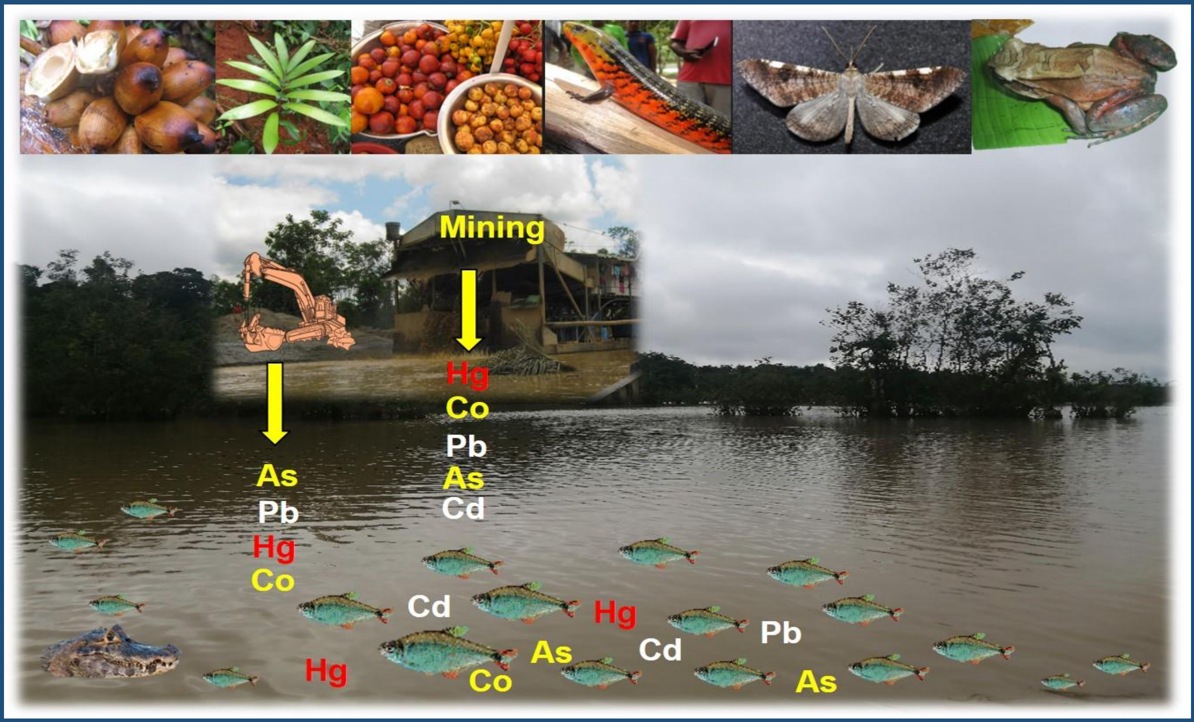
DOCTORAL THESIS

Environmental impact of toxic metal(loids) contamination in a tropical river impacted by gold mining in the Pacific region, Colombia



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Contents

General Abstract

Mercury (Hg) and arsenic (As) are two non-essential elements of global concern due to their toxicity and effects on the environment and human health. Methylmercury (MeHg), for example, is the most toxic organic Hg species classified as a potent neurotoxin capable of imposing irreversible effects on organisms. It is also the most common species present in aquatic environments. Other elements such as selenium (Se), although essential for organisms, can also cause toxic effects in high doses. Additionally, it has been reported that this metalloid can exert an antagonistic effect against Hg. All these elements occur naturally in the environment and their concentrations do not represent a risk in themselves.

However, the natural cycle of chemical elements present in the earth's crust has been strongly affected by anthropogenic factors including industrial activities, mining, agriculture and deforestation, facilitating the release of large quantities of toxic metals and metalloids into the environment. Once released into the environment, they can reach water sources and become trapped in the water column, in sediments and then bioaccumulated by aquatic organisms, mainly fish. In addition, they can biomagnify through the food chain and affect humans.

The purpose of this thesis is to evaluate anthropogenic Hg, As and Se contamination and the toxic effects induced in aquatic environments. Through a broad review of the available literature, in a first chapter we investigated the drivers of Hg, As and Se biomagnification in aquatic systems and the effects on wildlife. We also collected evidence of the antagonistic action of Se towards Hg. In this chapter we report that Hg has a higher biomagnification potential than As and Se. The slope of the trophic increase of Hg is consistent among different aquatic environments namely temperate (0.20), tropical (0.22) and arctic (0.22). In addition to the biology and physiology of the organisms, biomagnification of Hg, As and Se is highly dependent on environmental (e.g. latitude, temperature and physicochemical characteristics of the environment) and ecological (e.g. composition of the trophic structure and feeding zone of the organisms) factors. There is evidence

that Se exerts a mitigating role against Hg toxicity, but the maximum and minimum desired concentrations for this metalloid to activate its neutralizing action are unknown.

In the second chapter, we analyzed Hg, MeHg and As contents in water, sediment and fish samples collected from bogs in the Atrato River basin. The mean concentrations of Hg, MeHg and As in fish were 195.0 µg/kg, 175.5 and 30.0 µg/kg, respectively. In addition, the highest percentage of MeHg (89.7) was observed in the most consumed species, suggesting health risk for riparian communities. In sediment Hg (165.5 µg/kg), MeHg (13.8 µg/kg) and As (3.1 µg/kg), and in water Hg (154.7 ng/L) and As (2.1 ng/L). Thirty-eight percent of the total (n=205) of fish samples showed Hg concentrations above the limit concentrations for population protection set at 200 µg/kg, and 10% exceeded the limit for human consumption set at 500 µg/kg. On the contrary, As concentrations in fish were below the limit of 1000 µg/kg.

In water, Hg exceeded the threshold effect concentrations (12 ng/L), while As concentrations were below the threshold effect value (10,00 ng/L). According to nuclear alterations in fish, the species *Prochilodus magdalenae* showed higher response to the genotoxic damage categories evaluated, cells with nuclear budding (CBN, CNB, 3.7 ± 5.4%), micronuclei (MN, 1.6 ± 2.5%), binucleated cells (BC, 1.6 ± 2.3%).

In the third and final chapter, we evaluated the ecological and human health risk from exposure to contaminated sediments in the Atrato River basin. Hg levels in the sediments ranged from 0.09 - 0.23 mg/kg, and 0.59 - 2.68 mg/kg for As. From the Hg and As concentrations we used different ecological risk indices, namely CF (contamination factor) and PLI (pollutant loading index). We also use PERI (potential ecological risk index) and I_{geo} (geoaccumulation index). The HI (hazard index) and TLCR (total lifetime cancer risk) were used to assess the non-carcinogenic and carcinogenic risk, respectively.

According to the CF, most sediments were moderately contaminated with Hg and As at all sampling stations. The Igeo also suggested a moderate level of contamination for Hg and low for

As, the latter with an increasing trend. In contrast, the PLI revealed a progressive deterioration at one of the sampling stations (station B). When we compared Hg concentrations with the threshold effect concentrations (TEC) set at 0.18 mg/kg, we found that at stations A (0.23 Hg mg/kg) and C (0.20 Hg mg/kg) these were higher than TEC, indicating a toxic risk to aquatic biota, while As concentrations were lower than TEC indicating low risk to biota. The PERI detected a moderate risk at stations D and E, and a high risk for stations A, B and C.

The HI values were greater than 1, suggesting a high probability of occurrence of non-cancer adverse effects, particularly in children. In contrast, all values for TLCR were in the acceptable risk range of 1×10^{-6} and 1×10^{-4}, indicating insignificant cancer risk. However, oral ingestion and inhalation were identified as the two main routes of concern.

Overall, in this thesis we conclude that anthropogenic chemical pollution exerts a silent pressure on organisms. In addition, it considerably affects entire ecosystems, putting biodiversity and human well-being at risk. The conservation of aquatic fauna, mainly predatory organisms, is compromised by the presence of highly toxic substances such as Hg and As. Biomagnification of toxic pollutants is a critical variable to be included in environmental management policies, oriented towards environmental impact assessment and continuous monitoring of pollution in aquatic environments mainly.

Hg and As contamination of anthropogenic origin in the study area is evident, which could compromise local biodiversity and human health. In this sense, the results presented in this thesis should serve as inputs to strengthen environmental management policies at global and local scales. We also hope that these results will encourage researchers in the area to conduct studies to further evaluate the effects of toxic metal and metalloid contamination on biodiversity in the region.

Keywords: Atrato River, mining, mercury, arsenic, biomagnification, sediments, fishs, micronucleus, evaluación del riesgo.

Resumen General

El mercurio (Hg) y el arsénico (As) son dos elementos no esenciales de preocupación mundial, debido a su toxicidad y efectos en el ambiente y la salud humana. El metilmercurio (MeHg) por ejemplo, es la especie de Hg orgánico más tóxica catalogada como una potente neurotoxina capaz de imponer efectos irreversibles en los organismos. Además, es la especie más común presente en los ambientes acuático. Otros elementos como el selenio (Se) a pesar de ser esencial para los organismos, en altas dosis también puede provocar efectos tóxicos. Adicionalmente, se ha informado que este metaloide puede ejercer un efecto antagónico contra el Hg. Todos estos elementos se encuentran de forma natural en el ambiente y sus concentraciones no representan un riego en sí mismas.

Sin embargo, el ciclo natural de los elementos químicos presentes en la corteza terrestre, se ha visto fuertemente afectado por factores antropogénicos incluyendo actividades industriales, minería, agricultura y deforestación, facilitando la liberación de grandes cantidades de metales y metaloides tóxicos al ambiente. Una vez se liberan al ambiente pueden llegar a las fuentes hídrica y quedar atrapados en la columna de agua, en los sedimentos y luego ser bioacumulado por organismos acuáticos principalmente peces. Además, pueden biomagnificarse a través de la cadena alimentaria y afectar a los humanos.

En esta tesis nos planteamos como propósito evaluar la contaminación por Hg, As y Se de origen antropogénico y los efectos tóxicos inducidos en ambientes acuáticos. A través de una revisión amplia de la literatura disponible, en un primer capítulo nos ocupamos de investigar sobre los factores impulsores de la biomagnificación del Hg, As y Se en sistemas acuáticos y los efectos en la vida silvestre. También recogimos evidencias de la acción antagónica del Se hacia el Hg. En este capítulo informamos que el Hg tiene mayor potencial de biomagnificación a diferencia del As y Se. La pendiente del aumento trófico del Hg es consistente entre los diferentes ambientes acuáticos a saber, templados (0.20), tropicales (0.22) y árticos (0.22). Además de la biología y la fisiología de los organismos, la biomagnificación del Hg, As y Se depende en gran medida de factores ambientales (p. ej., latitud, temperatura y características fisicoquímicas del entorno) y

ecológicos (p. ej., composición de la estructura trófica y zona de alimentación de los organismos). Existen evidencias en donde él Se ejerce un papel mitigador contra la toxicidad de Hg, pero se desconocen las concentraciones máximas y mínimas deseadas para que este metaloide pueda activar su acción neutralizadora.

En el segundo capítulo analizamos contenidos de Hg, MeHg y As en muestras de agua, sedimentos y peces recogidas en ciénagas en la cuenca de Río Atrato. Las concentraciones medias de Hg, MeHg y As en peces fueron 195.0 µg/kg, 175.5 y 30.0 µg/kg, respectivamente. Además, el mayor porcentaje de MeHg (89,7) se observó en las especies de mayor consumo, sugiriendo riesgo para la salud para las comunidades ribereñas. En sedimentos Hg (165.5 µg/kg), MeHg (13.8 µg/kg) y As (3.1 µg/kg), y en agua Hg (154.7 ng/L) y As (2.1 ng/L). El 38% del total (n=205) de muestras de pescados mostraron concentraciones de Hg por arriba del límite de concentraciones para la protección de la población fijado en 200 µg/kg, y el 10% superó el límite para consumo humano establecido en 500 µg/kg. Al contrario, las concentraciones de As en pescado estuvieron por debajo del límite de 1000 µg/kg.

En agua el Hg excedió las concentraciones de efecto umbral (12 ng/L), mientras que las concentraciones de As estuvieron por debajo de su valor de efecto umbral (10,00 ng/L). De acuerdo con las alteraciones nucleares en peces, la especie *Prochilodus magdalenae* mostró mayor respuesta a las categorías de daños genotóxicos evaluadas, células con brotes nucleares (CBN, CNB, 3.7 ± 5.4%), micronúcleos (MN, 1.6 ± 2.5%), células binucleadas (BC, 1.6 ± 2.3%).

En el tercer y último capítulo evaluamos el riesgo ecológico y para la salud humana derivado por la exposición a sedimentos contaminados en la cuenca del Río Atrato. Los niveles de Hg en los sedimentos estuvieron en un rango de 0.09 - 0.23 mg/kg, y 0.59 - 2.68 mg/kg para As. A partir de las concentraciones de Hg y As empleamos diferentes índices de riesgo ecológicos a saber, por sus siglas en inglés: CF (factor de contaminación) y PLI (índice de carga de contaminante). También usamos el PERI (índice de riesgo ecológico potencial) y el Igeo (índice de geoacumulación). El HI (índices de peligrosidad) y el TLCR (riesgo de cáncer total de por vida) fueron usados para la evaluar el riesgo no carcinogénico y carcinogénico, respectivamente.

De acuerdo con el CF, la mayoría de los sedimentos estaban moderadamente contaminados con Hg y As en todas las estaciones de muestreo. El Igeo también sugirió un nivel de contaminación moderada para Hg y baja para As, esta última con tendencia creciente. Por el contrario, el PLI reveló un deterioro progresivo en una de las estaciones de muestreo (estación B). Cuando comparamos las concentraciones de Hg con las concentraciones de efecto límite o efecto umbral (TEC) establecidas en 0.18 mg/kg, encontramos que en las estaciones A (0.23 Hg mg/kg) y C (0.20 Hg mg/kg) estas fueron superiores a TEC, indicando un riesgo tóxico para biota acuática, mientras que las concentraciones de As fueron inferiores a TEC indicando bajo riesgo para la biota. El PERI detectó un riesgo moderado en las estaciones D y E, y un riesgo alto para las estaciones A, B y C.

Los valores de HI fueron mayores a 1, sugiriendo alta probabilidad de ocurrencia de efectos adversos no cancerígeno situado particularmente en los niños. Por el contrario, todos los valores para TLCR se situaron en el rango de riesgo aceptable de 1×10^{-6} y 1×10^{-4}, indicando riesgo de cáncer poco significativo. Sin embargo, la ingestión oral y la inhalación se identificaron como las dos rutas principales de preocupación.

De manera general, en esta tesis concluimos que la contaminación química antropogénica ejerce una presión silenciosa sobre los organismos. Además, afecta considerablemente ecosistemas enteros, poniendo en riesgo la biodiversidad y el bienestar humano. La conservación de la fauna acuática principalmente los organismos depredadores, se encuentran comprometida por la presencia de sustancia altamente tóxicas entre ellas el Hg y As. La biomagnificación de contaminantes tóxicos se constituye como una variable critica a incluir en las políticas de gestión ambiental, orientadas hacia la evaluación de impacto ambiental y monitoreo continuo de la contaminación en ambientes acuáticos principalmente.

La contaminación por Hg y As de origen humana en el área de estudio es evidente, lo cual podría comprometer la biodiversidad local y la salud de las personas. En ese sentido, los resultados presentados en esta tesis deberían servir de insumos para reforzar las políticas de gestión ambiental a escala global y local. Esperamos también que estos resultados alienten a investigadores de la zona especialmente, a realizar estudios que se ocupen por evaluar a mayor profundidad los efectos que impone la contaminación por metales y metaloides tóxicos en la biodiversidad de la región.

Palabras clave: Río Atrato, minería, mercurio, arsénico, biomagnificación, sedimentos, peces, micronúcleos, evaluación del riesgo.

General Introduction

Background

Freshwater ecosystems are threatened by many anthropogenic factors, including agriculture and mining, which compromise biodiversity (Aldana-Domínguez et al., 2019; Badrzadeh et al., 2022; Johnson et al., 2020; Reid et al., 2020). Mining in particular is one of the anthropogenic factors of greatest concern, due to the capacity of this activity to impose environmental changes on a planetary scale (Fernández-Lozano et al., 2020; Luo et al., 2020).

Alteration of natural cycles (e.g. hydrological and geochemical), loss of original vegetation cover, changes in landscape structure, and contamination with toxic substances are some of the most critical impacts associated with mining (Bose, 2023; Hogue & Breon, 2022; Martins-Oliveira et al., 2021; Rocha et al., 2020).

It is known that mining involves the removal of large volumes of earth, which facilitates the release of toxic elements into the environment, such as mercury (Hg), arsenic (As), lead (Pb) and cadmium (Cd) (Abende et al., 2023; de Almeida-Rodrigues et al., 2019; Marrugo-Negrete et al., 2018; Paschoalini & Bazzoli, 2021). Methylmercury (MeHg), for example, is the most toxic organic Hg species with the capacity to cause critical effects on organisms. It is also the most common form found in aquatic environments (Pang et al., 2022; Wu et al., 2022).

Once these elements are released into aquatic environments, they can easily enter the fish through the gills and be carried into the digestive tract and accumulate in major organs such as kidneys and liver. Consequently, the progressive bioaccumulation of these elements can cause metabolic and physiological changes and reduce the enzymatic capacity of the organisms, putting the sustainability of the species at risk (Dane & Şişman, 2020; Zaynab et al., 2022).

In this regard, a study has reported that of the 20,784 species of fauna (terrestrial and aquatic) for which the IUCN (International Union for Conservation of Nature) had data available, at least

18% of their conservation is compromised due to the effects of anthropogenic pollution (Hogue & Breon, 2022), including contamination with Hg, As, Cd and Pb (Abende et al., 2023; Becher et al., 2022).

The effects on the environment and human health caused by these metal(loids) are related to toxicity, persistence, and capacity for bioaccumulation and biomagnification (Ali & Khan, 2018b, 2018a). The latter refers to the increase in concentration of a contaminant as it moves from a lower to a higher trophic level (Azevedo et al., 2021; Newman, 2015; Szteren et al., 2023; Thanigaivel et al., 2023). Moreover, the effects are often silent and significantly impact the ecological dynamics of an entire ecosystem (Hamilton et al., 2017; Rodrigues-Filho et al., 2023; Saaristo et al., 2019).

For example, it has been reported that in polluted environments organisms invest much of their energy trying to reduce the chemical pressure caused by a given pollutant. They do this through a process known as biodilution. In other words, the unnecessary energy expenditure of an organism trying to adapt to stressful conditions, instead of investing it in other needs e.g. feeding and reproduction, could seriously compromise the viability of many wildlife communities (Hamilton et al., 2017; Hayase et al., 2010; Johnson et al., 2020).

Researchers and world authorities on environmental health, including the World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA), noted that despite warnings, controlling poisoning in the aquatic environment remains a difficult task, and the risks to animal and human health are increasing over time (Javed & Usmani, 2019; Jiang et al., 2023; Marrugo-Negrete et al., 2020; Pang et al., 2022; Paschoalini & Bazzoli, 2021; USEPA, 2023b; WHO, 2001).

However, despite calls for warnings, the problem of contamination of aquatic systems has received little attention in biological conservation studies (Sigmund et al., 2023). Even as trivial as it may seem, public health studies often overlook this situation as well (Groh et al., 2022;

Rodrigues-Filho et al., 2023). It is also noteworthy that concentrations of Hg and As, in particular, are increasing in almost all aquatic ecosystems of the world (Pang et al., 2022; UNEP, 2017; Wang et al., 2023), but also global efforts to reduce Hg emissions around the 1970s had positive effects mainly in industrialized countries, which was largely due to the technological changes made (Muntean et al., 2014). In contrast, this did not occur in developing countries, where mercury emissions grew considerably in the absence of technological alternatives to help control and reduce emissions (PNUMA, 2005).

Consequently, studies on the subject (e.g. original results, reviews and opinions) are gaining special relevance worldwide. This is partly due to the need to document the state of the problem at different sites in order to identify efficient measures to protect biodiversity and human health (Córdoba-Tovar et al., 2022; de Medeiros Costa et al., 2023; Galeano-Páez et al., 2021; Marrugo-Madrid et al., 2022; Paschoalini & Bazzoli, 2021; Sigmund et al., 2023; Xiao et al., 2022; Zaynab et al., 2022).

National and local context

In Colombia, gold mining is one of the main sources of economic income in the territories (Massé & McDermott, 2017; Perdomo & Furlong, 2022). In general, mining is carried out in a small-scale artisanal-ASM manner throughout the national territory (Cordy et al., 2015; Perdomo & Furlong, 2022), but it is also carried out with heavy machinery using backhoes and draglines, the latter being of greatest concern, due to the associated social and environmental impacts (Palacios-Torres et al., 2018; Veiga & Marshall, 2019).

Hg contamination is one of the main socio-environmental problems linked to the mining sector throughout Colombia (González-Álvarez et al., 2023; Marrugo-Negrete et al., 2020, 2021; Suárez-Criado et al., 2023). For example, Cordy et al., (2011) reported that about 50% of the Hg used in ball mills is lost, as well as 46% in the washing process, and 4% during amalgamation. The researchers pointed out that atmospheric emissions of Hg in 2011 reached 150 tons/year, data that placed Colombia as the country with the highest per capita Hg contamination in the world.

It has also been estimated that in the department of Antioquia alone, at least 90 tons of Hg are emitted annually into the air, water and soil (Cordy et al., 2015).

Moreover, this activity is often catalogued as a conflictive, bureaucratic and submerged economy (Jonkman, 2021; Jonkman & de Theije, 2022; Veiga & Marshall, 2019), mainly in territories where state control is weak or almost null (Jonkman & de Theije, 2022). In this regard, more recent data on gold production has revealed that for 2016 gold production reached 61.8 tons, where 87% of the gold was produced in informal mines and 13% in legally constituted mines (Veiga & Marshall, 2019).

At the same time, it should be noted that the impacts associated with mining are largely mediated by the increase in the price of gold. Thus, the higher the price of gold, the higher the use of Hg, which translates into more pollution (Grueso, 2019; Jonkman, 2022), and the practice of mining in biodiversity hotspot territories (Palacios-Torres et al., 2018; Paz, 2020), and in some cases in protected areas (UNODC, 2016). In addition, gold production in Colombia is expected to increase from 1.8 Moz in 2018 to 2.3 Moz by 2027 (Betancur-Corredor et al., 2018). This scenario poses risks for the conservation of the environment (Correa et al., 2020; Valois-Cuesta & Martínez-Ruiz, 2016), but also poses substantial impacts on the well-being of communities, due to the environmental degradation that may result from gold mining (Clavijo & Montaño, 2022; Gamboa-García et al., 2020; González-Álvarez et al., 2023).

For example, according to the national health institute-INS between 2013 and 2015 in Colombia, 1,126 cases of Hg poisoning were registered. Most cases were concentrated in territories with high mining influence among them the departments of Sucre (143), Bolivar (167), Córdoba (206), Chocó (218) and Antioquia (312) (Gaviria, 2016). Additionally, 76% of the total satellite detections of environmental degradation of the entire national territory are concentrated in the departments of Antioquia (37%) and Chocó (39%), being the territories with the largest environmental footprint derived from gold mining (UNODC, 2016).

In the study area, department of Chocó, the impacts of mining are imminent (Caicedo-Rivas et al., 2023; Marrugo-Negrete et al., 2023). For example, it has been reported that mining exerts strong pressure on natural ecosystems, to the point that it puts the sustainability of ecosystems at risk. Municipalities such as Condoto (9.43%), Istmina (7.75%), Novita (7.74%), Quibdó (7.64%) and San José del Palmar (6.56%) are territories that show a high rate of potential vulnerability to biodiversity loss (Valois-Cuesta & Martínez-Ruiz, 2016).

In addition, mining puts the biodiversity of the territory at risk (Betancur-Corredor et al., 2018), and is impacting people's health, due to contamination with Hg, Cd, Pb and As (Salazar-Camacho et al., 2022). For example, Hg concentrations in human hair of 17.54 ppm (Salazar-Camacho et al., 2017) and 116.4 ppm for the same matrix (Palacios-Torres et al., 2018), and in urine (23.89 µg/L) and blood (11.29 µg/L) samples (Gutiérrez-Mosquera et al., 2018). Along these lines, it has been reported that most of the fish species that are highly consumed by the population have concentrations of Hg, Pb, As and Cd above safe levels. Consequently, the population experiences a significant health risk, which has been revealed through carcinogenic risk indicators-CR (Salazar-Camacho et al., 2022) and hazard coefficient-HQ (Palacios-Torres et al., 2020).

Considering the environmental challenges posed by chemical contamination, and the need for knowledge, we highlight some of the contributions of this research that could be useful for environmental and public health authorities: (1) we provide synthesized information that allows a better understanding of the trophodynamics of Hg and As, critical effects and relevant factors involved in the biomagnification of these elements through trophic networks, (2) we explore for the first time genetic material alterations in freshwater fish in a tropical river protected by legal rights and their relationship with Hg and As concentrations in the Colombian Pacific, (3) This work also reports on the participation of MeHg in the Atrato River basin, a critical aspect to take into account for pollution monitoring and control, as well as for decision making in order to reorient environmental and public health policies. In this work we evaluated concentrations of Hg and As, since previous studies in the study area have shown that these two elements are of great concern.

However, we are unaware of the social and environmental concern imposed by other toxic metals including lead (Pb), cadmium (Cd) and copper (Cu).

Questions, hypotheses and objectives

Questions

¿How environmental and biological factors influence the biomagnification process of Hg and As in aquatic ecosystems?

¿What are the contents of Hg, MeHg and As in water, sediments and fish, and what toxic effects do they impose on fish in the Atrato River basin?

¿What levels of ecological and human health risk are posed by exposure to contaminated sediments in the Atrato River basin?

Hypotheses

Biomagnification of Hg and As in aquatic systems depends solely on the physicochemical characteristics of the environment.

The concentrations of Hg, MeHg and As reflect the impact of mining in the Atrato River basin and induce genotoxic damage in fish.

Exposure to sediments contaminated with Hg and As in the Atrato River basin puts the ecosystem's biota and human health at risk.

Objectives

General

Evaluate Hg and As contamination in environmental compartments (water, sediments and fish) in the Atrato River basin.

Specifics

To review the factors driving the biomagnification of Hg, As and Se in food webs in aquatic ecosystems.

Determine the concentrations of Hg, MeHg and As in water, sediments and fish, and the toxic effects on fish in the Atrato River basin.

Establish the ecological and human health risk of exposure to sediments contaminated with Hg and As in the Atrato River basin.

Overview of thesis structure

The present research thesis consists of three chapters corresponding to three of the three proposed objectives. Prior to the defense of this thesis the three chapters were published in the high impact international journal “Environmental Research Journal”. Each chapter is presented in a similar way to the journal format with its respective DOI (digital object identifier).

Chapter 1: **Drivers of biomagnification of Hg, As and Se in aquatic food webs: A review.** In this chapter we conduct a comprehensive review of the available literature on biomagnification of Hg and As in aquatic food webs on a global scale. Because it has been reported that Selenium (Se) can exert an antagonistic effect on Hg, here we also inquire about this relationship (i.e. Se:Hg), and trends in Se biomagnification in food webs. In general, this chapter highlights and discusses the relevant factors that lead to biomagnification of these metal(loids). In addition, we document the main effects that Hg, As and Se can have on wildlife, and present a list of fauna threatened by environmental contamination.

Chapter 2: **Toxic metal(loids) levels in the aquatic environment and nuclear alterations in fish in a tropical river impacted by gold mining.** Mainly in this chapter, Hg, MeHg and As contents were quantified in different environmental compartments (water, sediments and fish) in swamps in the Atrato River basin. The impact of fish life form on Hg and As bioaccumulation was evaluated. In addition, nuclear alternations in fish and their possible relationship with the concentrations of the analyzed pollutants were evaluated.

Chapter 3: **Ecological and human health risk from exposure to contaminated sediments in a tropical river impacted by gold mining in Colombia.** Finally, in this chapter we evaluate the ecological and human health risk derived from exposure to contaminated sediments in the Atrato River basin. Sediment quality is evaluated with respect to international sediment quality guidelines, and potential toxic risks to wildlife. We also estimate the risk of adverse non-carcinogenic and carcinogenic effects that could be experienced by the riverine population.

In terms of knowledge, this thesis provides information to help elucidate the toxicological risks imposed by Hg and As on the biota in the Atrato River basin, but also provides clues on critical points where environmental authorities should redouble their efforts to manage biological conservation and protect human health. Therefore, the results of this thesis support decision making in terms of strengthening and/or building environmental policies at local and national scales. For example, the three types of biomarkers (MN, CNB and BC) used in this thesis is an essential contribution to the detection of toxic effects imposed by chemical pollution. Moreover, it constitutes an important tool for environmental policy makers to manage and control pollution in the territories.

Chapter 1. Drivers of biomagnification of Hg, As and Se in aquatic food webs: A review

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Abstract

Biomagnification of trace elements is increasingly evident in aquatic ecosystems. In this review we investigate the drivers of biomagnification of mercury (Hg), arsenic (As) and selenium (Se) in aquatic food webs. Despite Hg, As and Se biomagnify in food webs, the biomagnification potential of Hg is much higher than that of As and Se. The slope of trophic increase of Hg is consistent between temperate (0.20), tropical (0.22) and Arctic (0.22) ecosystems. Se exerts a mitigating role against Hg toxicity but desired maximum and minimum concentrations are unknown. Environmental (e.g. latitude, temperature and physicochemical characteristics) and ecological factors (e.g. trophic structure composition and food zone) can substantially influence the biomagnification process these metal(loids). Besides the level of bioaccumulated concentration, biomagnification depends on the biology, ecology and physiology of the organisms that play a key role in this process. However, it may be necessary to determine strictly biological, physiological and environmental factors that could modulate the concentrations of As and Se in particular. The information presented here should provide clues for research that include under-researched variables. Finally, we suggest that biomagnification be incorporated into environmental management policies, mainly in risk assessment, monitoring and environmental protection methods.

Introduction

Anthropogenic sources of pollution

Freshwater aquatic ecosystems (e.g. lakes, streams, rivers, wetlands and reservoirs) host at least 10% of the world's known fauna including 30% of all vertebrates and play an important role in the global water cycle (Reid et al., 2020). Unfortunately, freshwater fish populations have declined by 83% between 1970 and 2014 as a result of human activities (Reid et al., 2019). In the last 50 years the extraction of resources (minerals, fossil fuels, biomass, soil, wood and water) has increased from 27 to 92 billion tons due to the consumption and demand of the world population, being one of the main causes of 90% of the loss of biodiversity and global environmental pollution (Oberle et al., 2019). Added to this is the lack of commitment of many countries to strengthen policies for the conservation of aquatic ecosystems, which makes this an extremely complex problem (Bland et al., 2019; Reid et al., 2020).

It is well known that anthropogenic activities alter the natural dynamics of metals increasing their availability in aquatic systems in particular (Crespo-Lopez et al., 2021; Vareda et al., 2019). Mining, agriculture, forestry, and deforestation are among those activities that favor increased concentrations of metals such as Hg and metalloids such as As and Se. Both Hg, As and Se are contributed to the environment naturally, however, their increase in the environment has resulted from the alteration of its cycle as a consequence of intensive anthropogenic activities (Johnson et al., 2020; Kato et al., 2020; Kozak et al., 2021; Ziyaadini et al., 2017). Overall, total global Hg emissions to the atmosphere range from 6500 to 8200 Mg yr^{-1}, of which approximately 35% are from direct anthropic sources (Driscoll et al., 2007; Mason & Sheu, 2002).

Toxicity to wildlife and humans

Contamination by Hg and As is a global threat because they are among the toxic elements for human and wildlife (Cardwell et al., 2013; Ziyaadini et al., 2017). Once they enter the aquatic environment become accumulated mainly by fish (Crespo-Lopez et al., 2021; Kato et al., 2020).

Fish often top the list of the main recipient organisms of toxic substances, constituting a risk to human health as the main protein consumed in many regions of the world (Azevedo et al., 2021; Garvey, 2019). Even at low concentrations Hg and As have the ability to biomagnify through the food chain can cause adverse effects on organisms (Jasonsmith et al., 2008; Newman, 2015; Ventura-Lima et al., 2011).

Biomagnification and effects to biota and human

The effects on the environment and human health are related to toxicity, persistence and ability to increase their toxic potential as they move from a lower to a higher trophic level, known as biomagnification (Hallare et al., 2011; Newman, 2015). In wild organisms the effects include decreased reproductive capacity, reduced embryo viability, teratogenesis and reduced enzymatic processes (Kumari et al., 2017; Zheng et al., 2019). As can disrupt the growth of plankton, crustaceans and mollusks, and decrease photosynthetic processes affecting ecological processes and productivity of aquatic ecosystems (Barwick & Maher, 2003; Newman, 2015).

In relation to Se, it has been shown that excessive levels can cause deformities in fish, birds and mammals (Johnson et al., 2020). In humans, the accumulation of Hg above the tolerance limit affects the central nervous system, generates cytogenic damage, cardiovascular problems, mental retardation and renal dysfunction (USEPA, 2017). According to the World Health Organization, in fishing regions of countries such as Colombia, China, Brazil and Greenland, 17 out of every thousand children suffer mental disabilities related to the consumption of fish contaminated with Hg (WHO, 2017). In relation to biomagnification, there are not many cases where increased concentrations of As and Se are reported at higher trophic levels of food webs (Cardwell et al., 2013; Hayase et al., 2010), while for Hg there is an ample evidence of biomagnification in different ecosystems (Campbell et al., 2008; Clayden et al., 2015; Lavoie et al., 2013). Anyway, knowledge gaps persist in terms of Hg biomagnification (Carrasco et al., 2011; Clayden et al., 2015). Therefore, part of the environmental debate on biomagnification of metals and metalloids is due to the fact that the particular and intrinsic characteristics of the ecosystems involved in this process are not fully understood (Espejo et al., 2020; Gray, 2002; Ikemoto et al., 2008; Lavoie et al., 2013).

With respect to Se there is an important antagonism, since it has been reported to have a reducing effect against MeHg toxicity. It is believed that the effect is related to the ability to modify the biokinetic processes of Hg, exerted by the action of selenomethionine, selenocystine and selenite. However, this remains a subject of debate, controversy and research (Amlund et al., 2015; Bjerregaard et al., 2011; Bjerregaard & Christensen, 2012; Luo et al., 2020). Additionally, it has been suggested that the reductive effect may also be related to chemical characteristics, trophic structure, ecological processes (Arcagni et al., 2017) and specific detoxification mechanisms developed by some organisms in particular fish (Arcagni et al., 2013; Ikemoto et al., 2008). This study aimed to explore relevant factors driving the biomagnification of Hg, As and Se in aquatic food webs. In addition, special attention was paid to the negative impacts on biota (e.g. birds, fish and marine mammals). The study is based on the relevance of generating useful information to help those responsible for developing environmental management policies to manage future challenges in terms of biota conservation and health protection.

Method

We conducted an orderly literature search on biomagnification of metal(loids). Four databases were reviewed. Scopus (https://www.scopus.com), Wiley Online Library (https://onlinelibrary.wiley.com), JSTOR (https://www.jstor.org) and ScienceDirect (Elsevier) (https://www.sciencedirect.com). The term "biomagnification of metals" was the key descriptor used in the information search. Additionally, "mercury", "arsenic" and "selenium" together with the combination of the terms "toxicity", "effects on human health", "effect on biota", “aquatic ecosystems" and "risk assessment" were also considered in the search and selection of papers (Figure S1).

In general, books, book chapters and technical reports from authorities on environmental health issues (USEPA and WHO) were considered. However, for the description of the factors leading to biomagnification of the selected elements (Hg, As and Se), only papers where biomagnification was evaluated based on isotopy ($\delta^{15}N$ and $\delta^{13}C$) and calculation of the trophic magnification factor, published in scientific journals from 2000 to April 2021, were considered. For the selection of papers, the quality and relationship of the information with the purpose of the particular study was considered. Research that reported on biomagnification in terrestrial ecosystems was not considered. In addition, a co-occurrence analysis of keywords used in the research and a global map of the network of collaborating countries were carried out. The programs VOSviwer 1.6 and R-Studio version 4.0 were used for these analyses. Finally, the conservation status of some of the fish, bird and marine mammal species reported in the studies reviewed was verified. For this purpose, the global database of the International Union for Conservation of Nature (IUCN) was consulted (https://www.iucnredlist.org/search?query=Phoca%20hispida&searchType=species).

Results and discussion

Scientific production related to biomagnification of metal(loids)

The term "biomagnification" refers to the increasing concentration of a contaminant as it moves up the food chain. If predatory organisms absorb a contaminant more than excrete and eliminate, they could have higher concentrations than their prey. Figure 1 shows the trophodynamics and biomagnification of metals in the aquatic environment. It has also been used to describe the enrichment of contaminants in food webs with respect to their trophic position including humans (Drouillard, 2008; Newman, 2015).

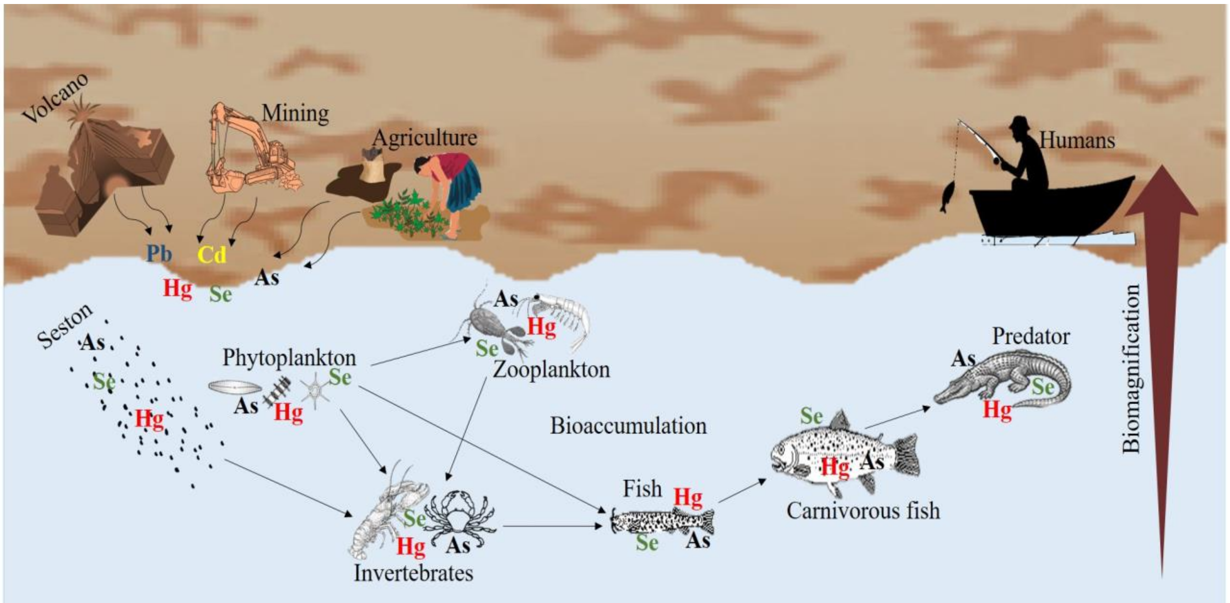

Figure. 1. Graphical illustration of trophodynamics and biomagnification of metals in aquatic food webs.

The interest in knowing about the biomagnification of contaminants is related to the effects on health and wild organisms, especially those of higher trophic levels (Mackay et al., 2016). The need to generate information becomes especially relevant after human poisoning by consuming fish contaminated with Hg in the city of Minamata, Japan, in 1956, known as Minamata disease (Harada, 1995). Later, in the 1960s, concern was extended to biota following findings of high concentrations of toxic substances in large predators and the gradual and unexplained decline of bird populations associated with aquatic ecosystems (Moriarty, 1990). This was largely mediated by the indiscriminate use at that time of seeds treated with mercurial fungicides for the control of crop diseases, with high implications on the ecosystem and human health (Soliman, 1956).

The first evidence of biomagnification was obtained after analyzing empirical data on the chlorinated insecticide dichloro diphenyl trichloroethane (DDT) in food webs composed of plankton-fish and birds in California's Clear Lake, which was a major milestone in environmental science. Since then, its application and validation were extended to other food webs with original data starting in 1980 (Drouillard, 2008).

A total of 11,864 documents (research and review articles) published from 2000 to April 2021 were registered among the four databases consulted, scopus (6,192), ScienceDirect (4,158), JSTOR (355) and Wiley Online Library (1,159). The bulk of the information has been published in the form of research articles 10,450 (88.0%) and review articles 1,414 (12.0%). It also shows a significant growth in publications over time (Figure S2). The highest number of publications occurred in the year 2020 with 693 publications equivalent to 11% of the overall production. This growth suggests that the subject has been gaining special relevance in the world.

There is a wide network of collaborating countries concerned with filling knowledge gaps on the dynamics of biomagnification of metal(loids) in food webs. This suggests that biomagnification has been of concern in many regions of the world. However, there are differences between countries in terms of scientific production, which shows the advances and lags in knowledge. Figure S3 shows the distribution of scientific production on biomagnification of metal(loids) in aquatic systems and the network of collaborating countries.

It was found that the research in the titles and summaries highlight a series of key words that not only attract attention, but also inform about the complexity of the problem that aquatic ecosystems are going through due to contamination. The most frequently occurring are fish, mercury, impact and toxicity. However, concentration and level are the words with the greatest weight. Figure S4 shows the 15 keywords with the highest occurrence in titles and abstracts with at least 5 occurrences in a set of 43,444 words and 11,864 documents (research and review articles).

Drivers of biomagnification of Hg, As and Se

Globally, some of the literature has indicated that biomagnification in aquatic systems is largely influenced by environmental, ecological and biological factors (Barwick & Maher, 2003; Campbell et al., 2008; Huang, 2016; Wyn et al., 2009; Zhang et al., 2012). Some studies reporting biomagnification of Hg, As and Se in aquatic systems (i.e. sea, rivers and lakes) are shown in Table S1.

Mercury

Hg is the main toxic metal of global concern. The reason why it has been widely studied has to do with toxicity, persistence, bioaccumulation, and because its health and environmental hazardous effects (Azevedo et al., 2021; Coelho et al., 2013). Methylmercury (MeHg) is the most toxic organic Hg species that reaches food webs and has the ability to biomagnify along food webs (Campbell et al., 2005; Crespo-Lopez et al., 2021; Du et al., 2021; Lavoie et al., 2010).

In small polynya ecosystems (open space surrounded by sea ice) of the Arctic, Canadá, biomagnification of Hg from copepods to fish with slope (increased $\delta^{15}N$ ratio) of 0.15 has been reported. MeHg concentrations were exceeded more than 50-fold from copepods (*Calanus hyperboreus*) to seabirds, particularly in terns (*Sterna paradisaea*). However, the slope had an increase of 0.26 when seabird chicks were included in the analyses (Clayden et al., 2015). In networks composed of fish, plankton and benthic invertebrates from Lake Ontario, Canadá, variations in Hg concentrations have been found with respect to seasonality, where concentrations tended to increase in spring and decrease in summer for most organisms. The values of the slope of increase for the seasons showed variations between 0.17 and 0.24 and 0.2 throughout the year. The biomagnification trend was observed especially in fish as a function of seasonal variation (Zhang et al., 2012).

Lipid content, trophic level, body weight and benthic connection explained total mercury (THg) and MeHg concentrations by 77.4 and 80.7%, respectively, in marine nets (plankton, invertebrates, fish and seabirds) from the Gulf of St. Lawrence, Canadá. Both THg and MeHg biomagnified with a slope (slope of the $\delta^{15}N$ ratio) of 0.17 for Hg and 0.23 for MeHg. The excess concentrations were more evident in pelagic and benthopelagic species as opposed to benthic species. This indicates that Hg may be readily bioavailable to benthic organisms (Lavoie et al., 2010).

Researchs have indicated that speciation, food preference, ecological seasonality and physicochemical characteristics (e.g. total phosphorus and total nitrogen) are among those typical factors with substantial influence on Hg biomagnification in temperate environments. Furthermore, the slope of increasing Hg concentrations in large high-latitude lakes follows a pattern consistent with global biomagnification rates (Chen et al., 2008; Jæger et al., 2009; Kidd et al., 2012; Zhang et al., 2012).For example, in estuarine environments of Masan Bay, southeast coast of Korea, THg and MeHg concentrations (%MeHg) ranging from 9.57 to 195 and 2.56 to 111 ng/g dry weight (dw) (12-86%) have been recorded in benthic invertebrates. In addition, benthic fish THg and MeHg (%MeHg) concentrations ranged from 10.8 and 618 ng/g and 2.90 and 529 ng/g dw (23–94%), respectively. The trophic magnification factor (TMF), expressed as the slope of the linear regression of contaminant concentrations on $\delta^{15}N$ values, were higher for benthic organisms, with values of 0.12 for THg and 0.17 for MeHg. The increase in concentrations was attributed to the physicochemical and biological characteristics of the ecosystem (Kim et al., 2012).

In contrast, in Laizhou Bay northern China, weight and ecotype were the factors that were related to Hg concentrations along the assessed food web (plankton, fish and invertebrates), and the TMF for MeHg and THg were 2.09 and 1.69, respectively (Cao et al., 2020). In general, the slope of biomagnification can vary with respect to climatic conditions. For example, it has been reported that the biomagnification rate is higher in cold ecosystems with low productivity. On average, the trophic magnification slope (TMS) for THg is 3.7 ± 0.8 and 3.8 ± 0.8 for MeHg. MeHg gain in freshwater food webs is 1.5 times higher than THg and shows increase with respect to dissolved organic carbon and decrease with total phosphorus and atmospheric decomposition (Lavoie et al., 2013). However, it appears that the results of THg and MeHg biomagnification estimates tend to be higher in high latitude environments compared to low latitude environments (Al-Reasi et al., 2007; Campbell et al., 2005; Lavoie et al., 2013; Poste et al., 2015; Seco et al., 2021). But variations in the values of increasing slope at higher trophic levels require further clarification among ecosystems of the world (Lavoie et al., 2013; Sun et al., 2020). Figure 2 shows the global variation of the slope of trophic increment in temperate and tropical systems.

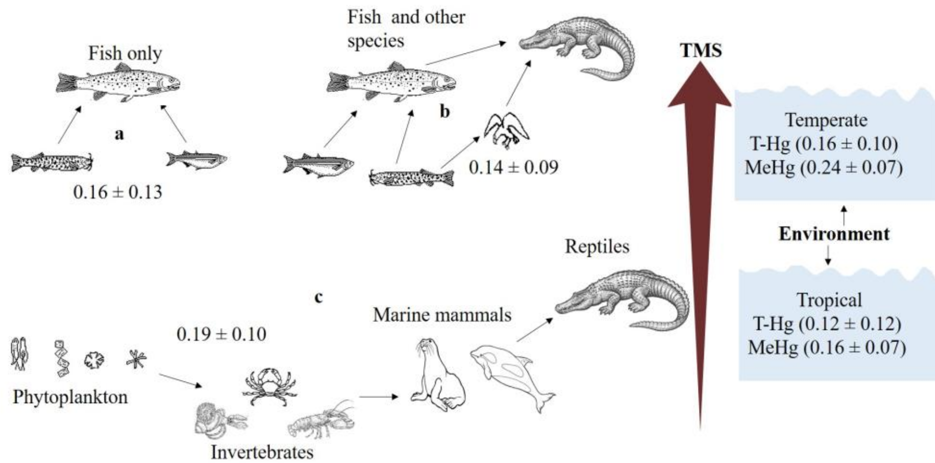

Figure. 2. Global variation of the increase of THg and MeHg in different environments and trophic structures. a = fish food web. b = foob web made up of fish and other species. c = complex foo web without the presence of fish. Concentrations are presented as mean ± standard deviation. Source: inspired in Lavoie et al., (2013).

A recent study reported that in food webs composed of marine macroinvertebrates, fish and seabirds from western Patagonia and the western Antarctic Peninsula, the slope of trophic increase increased when seabirds and marine mammals were excluded (0.13 to 0.22). Suggesting that the composition of the food web structure had an important influence on Hg biomagnification (Chiang et al., 2021). In general, it has been indicated that Hg may be biomagnifying at similar rates in the Great Lakes, and the slopes of increase appear to be consistent among different Antarctic, tropical and temperate ecosystems. For example, in a pelagic Artic marine food web (Baffin Bay) the slope is 0.22 (Campbell et al., 2005), similar than in the tropical African Lake Tanganyka 0.22 (Campbell et al., 2005, 2008), in the tropical Colombia Ayapel marsh 0.20 (Marrugo-Negrete et al., 2018), or in the Bay of Fundy located in the Gulf of Marine 0.20 (Harding et al., 2018) and Gulf of St. Lawrence 0.24 (Lavoie et al., 2010). In addition, the rate and power of MeHg biomagnification in tropical, subtropical, temperate and Arctic aquatic ecosystems is also favored by the methylation capacity possessed by some gut microbes present in zooplankton and by some methanotrophic bacteria contributed by invertebrates and fish (Harding et al., 2018; Pouilly et al., 2013). In tropical environments, periphyton associated with macrophyte roots is also a potential source of Hg methylation, and may even be a matrix with a higher rate of MeHg production than that produced in the sediments and water column

(Lázaro et al., 2016). This may in part be due to the diversity of communities of organisms present in the periphyton and complex microbial interactions (Achá et al., 2011). In that sense, the rate of Hg methylation in the aquatic environment is largely influenced by the productivity of anaerobic microorganisms and environmental conditions including pH, organic carbon and temperature, as well as the bioavailability of inorganic Hg (Joorabian et al., 2023).

Temperature is also another environmental factor that can explain Hg concentrations in food chains. In this regard, it has been suggested that there are several mechanisms that are related. One of them, and the most relevant, is that at warmer temperatures greater stimulation of the growth of aquatic organisms, and a lower amount of Hg per unit of body mass, unlike cold temperatures, in which growth is often suppressed. This suggests that the growth rate could modulate Hg concentrations (Lavoie et al., 2013; Poste et al., 2015; Simoneau et al., 2005; Tadiso et al., 2011). The influence of biological (morphological structure) and environmental (O_2 and pH) characteristics on Hg biomagnification has also been reported, as they are factors influencing Hg assimilation and mobility especially in macrofauna (Jędruch & Beldowska, 2020). For example, the age, sex, rapid growth of animals, as well as ontogenetic changes in diet, can affect MeHg concentrations. This is in part due to the fact that organisms as they reach maturity need to consume larger prey, which typically represents an increase in MeHg concentrations from the consumption of large contaminated prey (Chételat et al., 2020).

This supports the hypothesis that MeHg concentrations in aquatic food webs in general are the result of the combination of ecological dynamics influencing dietary exposure and physiological processes responsible for assimilation, transformation and elimination, and environmental factors particular to each area with an impact on contaminant mobility (Chételat et al., 2020; Jędruch & Beldowska, 2020; Kwon et al., 2012; Lemos-Bisi et al., 2012; Souza et al., 2020). Additionally, it has been suggested that in temperate freshwater lakes the level of sulfate in the water can enhance biomagnification, as it can have a strong, almost direct effect on Hg methylation (Gentés et al., 2021). However, in both temperate and tropical systems the level of bioaccumulation and biomagnification may be more dependent on the joint influence of temperature (warm), productivity (e.g. total phosphorus) of the ecosystem, food availability and the high influx of Hg-enriched organic matter (Al-Reasi et al., 2007; Clayden et al., 2015; Gentés et al., 2021; Jędruch et al., 2019; Kozak et al., 2021). This suggests that the drivers of Hg biomagnification in aquatic ecosystems in general are complex, diffuse, and point-wise at the same time (Azevedo et al., 2021; Clayden et al., 2013; Lescord et al., 2014; Murillo-Cisneros et al., 2019; Omara et al., 2015).

Arsenic

The presence of As in the aquatic environment is also a time bomb that threatens wildlife and human health (Kato et al., 2020). In marine and freshwater systems As is present as a mixture of arsenic and arsenate. The latter is the most prevalent As species in aquatic ecosystems (Cutter et al., 2001). However, little is known about trophodynamics in freshwater systems in particular (Caumette et al., 2012; Huang, 2016; Kumari et al., 2017; Tu et al., 2011; Yang et al., 2020). Naturally As concentrations in aquatic environments (rivers and lakes) range from less than 0.5 μg L^{-1} to more than 5000 μg L^{-1}. In freshwater systems typical concentrations are less than 10 μg L^{-1} and often less than 1μg L^{-1}. In addition, concentrations are determined by the availability, source and geochemistry of the water body (Azizur Rahman et al., 2012; Smedley & Kinniburgh, 2002). It is well known that periphyton and phytoplankton are the main food source for many organisms. But they also play an important role in As biotransformation, which favors bioaccumulation and trophic transfer (Azizur Rahman et al., 2012; Lopez et al., 2016). Excessive concentrations have often been reported in polychaetes, crustaceans and algae (Farías et al., 2007; Hayase et al., 2010; Waring & Maher, 2005). For example, concentrations between 4.70-5.65 μg g^{-1} have been found in the crustacean species *Aristeomorpha faliacea* (Hayase et al., 2010), polychaetes *Tharyx marioni* 1.5-2739 μg arsenic/dry mass (Waring & Maher, 2005) and in algae Myriogramme sp 5.8 μg g^{-1}, *Himantothallus grandifolius* 152 μg g^{-1} (Farías et al., 2007).

Regarding the potential for biomagnification in food webs, a variety of drivers have been reported, and also many of the results on bioaccumulation and biomagnification are inconsistent especially when it comes to demonstrating increased concentrations from algae to zooplankton, and from zooplankton to forage fish to predatory fish (Azizur Rahman et al., 2012; Kato et al., 2020; Maher et al., 2011; Majer et al., 2014). It has been reported that in freshwater systems the concentration level is related to the rate of biodilution as opposed to marine, which is more associated with the enrichment of the organic form (arsenobetaine) and the degradation of foods with high arsenosugar contents (Caumette et al., 2012; Huang, 2016; Kirby et al., 2002). In marine nets in Daya Bay, China, low concentrations of As were recorded in pelagic organisms as opposed to benthic organisms (Du et al., 2021). At high trophic levels (fish, shrimp, crabs and cephalopods) concentrations were lower in pelagic dietary fish in summer and higher for benthic fish in winter. This suggests that the transfer, bioaccumulation and biomagnification potential of As in marine environments may be favored by benthic habits and environmental factors (Du et al., 2021). In addition, it has been indicated that the negative effects may be greater in organisms with low trophic positions, possibly due to the effect of As biodilution along food webs (Hayase et al., 2010; Trevizani et al., 2018).

In deep waters of Sagami Bay, Japan in marine nets consisting of fish, crustaceans and zooplankton, low concentrations of total As were recorded in bottom fish (23.6 ± 14.6 μg g^{-1}; $p < 0.05$), pelagic fish (8.50 ± 5.66 μg g^{-1}) and zooplankton (8.93 ± 2.70 μg g^{-1}). Concentrations were also significant in crustaceans (17.0 ± 7.63 μg g^{-1}; $p < 0.05$). In addition, a significant increase in lipid-soluble As concentrations was observed especially in pelagic organisms. On the contrary, total As and arsenobetaine were biodiluted along the food web (Hayase et al., 2010). In tropical mangrove systems in southern Vietnam, biomagnification of As in food webs consisting of fish, crustaceans, octopods, plankton and cephalopods has also been reported. An increase in the slope of lipid-soluble As (0.13) and arsenobetaine (0.10) was observed indicating significant biomagnification in this food web (Tu et al., 2011). On the other hand, in the Derwent Estuary, south west of Tasmania, significantly higher high concentrations have been reported in seabirds with food preference in the lower part of the food web. *Haematopus longirostris* (1.67 ± 0.99 μg g^{-1}), *Phalacrocorax fuscescens* (1.2 ± 0.8 μg g^{-1}) and *Cygnus atratus* (1.0 ± 0.5 μg g^{-1}). These results account for the possible influence of feeding zone in modulating concentrations at higher trophic levels (Einoder et al., 2018). In other key predators such as marine turtle *Eretmochelys imbricata*, age may be a factor in helping to understand excess concentrations; however, accumulation may be species-specific (Agusa et al., 2008).

In addition, bioaccumulation and biomagnification tendency may vary with respect to the type and complexity of the food web (Dovick et al., 2015; Huang, 2016; Waring & Maher, 2005). High concentrations in some predators is not necessarily linked to trophic position and feeding habit, as retention capacity, assimilation, metabolization and exposure time may play an important role in bioaccumulation and biomagnification (Kirby et al., 2002; Maher et al., 2011).

In general, the physiology, food preference and metabolic capacity of organisms are the fundamental factors that explain and help to understand the trophodynamics and biomagnification of As in aquatic food webs (Kato et al., 2020; Kirby et al., 2002; Pouil et al., 2020). However, some authors have indicated that As does not biomagnify in the same way as Hg. This means that unresolved questions remain around trophodynamics, bioaccumulation and biomagnification particularly in freshwater systems (Furtado et al., 2019; Yang et al., 2020).

Selenium

Despite being an essential element excess concentration can cause adverse effects on biota (Jasonsmith et al., 2008; Savery et al., 2013). In the aquatic environment Se is introduced by plankton in particulate form, and therefore it is incorporated into organic matter and transferred from invertebrates to fish (Schneider et al., 2015). Globally, there is a growing interest in elucidating the results of Se and Hg interaction in aquatic ecosystems, as Se has been reported to exert a reducing effect against MeHg toxicity (Azad et al., 2019; Bjerregaard & Christensen, 2012; Burger et al., 2013b; Burger & Gochfeld, 2013a). Chemically, it is well known that Se has a significant affinity for Hg and is capable of exerting neutralizing action on the effects of MeHg. This action is usually indicated by the Se:Hg molar ratio, where ratio values greater than 1 indicate potential protection against Hg toxicity (Arcagni et al., 2017; Jasonsmith et al., 2008; McCormack et al., 2020; Økelsrud et al., 2016; Savery et al., 2013).

Often this molar ratio in food webs composed of pelagic fishes has variations in content ranging from 0.10 to 2.25. Probably the contents and variations may be related to ecological dynamics, variety in diet (Kaneko & Ralston, 2007) and geography of the territory (Azad et al., 2019). For example, it has been reported that in *Physeter macrocephalus* (sperm whale), a species of high trophic level and wide distribution, global Se concentrations are higher than those of Hg with a mean Se:Hg molar ratio of 59:1 (Savery et al., 2013). Furthermore, Hg:Se 1:1 molar ratios have been reported in several odontocete species, indicating that Se may be generating a protective effect against Hg toxicity in these large ocean mammals (McCormack et al., 2020). For fish, significant levels of Se have generally been found to correlate with length, and it is suspected that fish greater than 50 cm in length are less likely to be beneficiaries of the reducing effect as opposed to smaller fish (Burger et al., 2013b).

In relation to factors influencing biomagnification, trophic structure and diet have been reported to be key factors that help to understand trophodynamics, accumulation and biomagnification along food webs (Arcagni et al., 2013; Schneider et al., 2015). Studies have indicated that in networks composed of plankton, algae, invertebrates, macrophytes and fish, Se only biomagnifies in fish and its biomagnification potential could be related to the complexity of the food web (Arcagni et al., 2013; Jasonsmith et al., 2008; Mann et al., 2011; Økelsrud et al., 2016). However, it has been expected that biomagnifying potential is more evident in freshwater lakes and depends on the species chemistry (Arcagni et al., 2013; Dang & Wang, 2011; Jardine et al., 2006; Schneider et al., 2015). For example, in complex food webs (plankton, macroalgae, benthic microalgae, invertebrates, detritus, seagrasses, epiphytes and fish) from Lake Macquarie (New South Wales, Australia), Se concentrations ranged from 0.2 μg g^{-1} (macroalgae) to 12.9 μg g^{-1} in predatory fish and a mean magnification factor of 1.39 per trophic level, indicating a significant biomagnification. Feeding site in conjunction with habitat were the factors that may explain the magnification (Schneider et al., 2015).

The variety in the food diet in conjunction with the size, age and weight of the fish had an influence on the biomagnification of Se in nets of goldfish (*Coryphaena hippurus*) in the Gulf of

California, Mexico (Bergés-Tiznado et al., 2019). Values of Se:Hg molar ratios are 3.7 in muscle and 697.1 in gonads). Smaller fish did not show biomagnification (<80cm fork length), while larger ones (>90cm fork length) showed percentages of 100% for Hg and 65% for Se. Biomagnification has also been reported in sailfish (*Istiophorus platypterus*) nets in the eastern tropical Pacific one of the most productive oceans in the world, where Se:Hg molar ratios are higher than one. Maximum Se concentrations in kidneys (14.1 ± 1.9 μg g^{-1}) and minimum in muscles (0.67± 0.03 μg g^{-1}) were recorded indicating biomagnification of both elements in this food web (Bergés-Tiznado et al., 2015). Se and Hg concentrations showed variations between 0.41 mg Se kg^{-1} and 0.06 mg Hg kg^{-1} in invertebrates and 2.9 mg Se kg^{-1} and 3.6 mg Hg kg^{-1} in fish. The TMF for Se was 1.29 and 4.64 for Hg, indicating that both elements biomagnified through the network (Økelsrud et al., 2016).

Effects on aquatic biota

Metal(loids) contamination is undoubtedly a threat to wildlife and humans (Ruffle et al., 2019; Rumbold et al., 2018; WHO, 2017). This consideration becomes particularly relevant insofar as the effects on organisms lead to significant alterations of ecological and biological processes as a consequence of affecting habitat quality (Abbasi et al., 2015; Cobbina et al., 2015; Di Cesare et al., 2020).

Fish

Fish are one of the most impacted species by pollutants due to their relatively high trophic position in the aquatic ecosystem (Martins et al., 2020; Gray, 2002). One of the main effects of As is enzymatic reduction, hyperglycemia, and it substantially impacts the immune system (Kumari et al., 2017). In excess concentrations, Hg increases the risks of adverse effects among them: induction of teratogenesis, decrease in reproductive capacity, damage to tissues, genes, proteins and an apparently simple effect, no less important, is the effect on the ecological behavior (Zheng et al., 2019). In relation to Se, concentrations alone do not pose a serious problem for these organisms, however, when reacted with other metals, in particular Hg, it is likely that it can adversely affect the reproductive system in almost all aspects, e.g. decreased embryonic survival and fertility, overall reproductive success (Jasonsmith et al., 2008; Mann et al., 2011; Penglase et al., 2014), blood changes, reduced growth and mortality increase of juveniles (Fernández-Trujillo et al., 2021).

Birds

The bioaccumulation of Hg in seabirds and waterbirds species is also worrisome, because it affects the flight capacity and the health status of birds with different feeding habits (Ullah et al., 2014; Whitney & Cristol, 2017). This effect occurs because Hg has a high affinity to keratin in feathers and ramphoteca, rich in sulphur-containing amino acids (L-cysteine) (de Medeiros Costa et al., 2021). In this sense, blood Hg levels are influenced by feather growth and moulting (Condon & Cristol, 2009), therefore, depuration occurs more rapidly in molting birds and reduction of Hg concentrations can be up to 90% of the concentrations in the blood during the molting process (Whitney & Cristol, 2017). In contrast, in seabirds (e.g. *Rissa tridactyla*) Hg can affect gonadotropin-releasing hormone (GnRH) responsible for the release of sex steroids, compromising reproductive success in both sexes (Tartu et al., 2013). When considering the toxic effects of Se on birds, it is probably known that it can affect reproductive success due to weight reduction, egg hatching, deformities (e.g. defect of the beak, eyes, microphthalmia and anophthalmia) and induce hydrocephalus (Heinz et al., 1987). In relation to the effect of As, it has important effects on the health status of organisms, thus producing hematological effects (e.g. reduction in the production of red blood cells and hematocrits) (Geens et al., 2010) and endocrine disruption (Neff, 1997).

Marine mammals

The available literature on toxic effects on marine mammals appears to be less common compared to that available for other organisms. However, studies show that Hg toxicity produces adverse effects in this species that include damage to the reproductive, nervous and excretory systems (Wolfe et al., 1998). One of the most common effects of As reported in these animals is the induction of homeostasis imbalance (Ventura-Lima et al., 2011). Additionally, it is evidenced that inorganic As can act as an endocrine disruptor (Kunito et al., 2008). Finally, a study reports that excess Se concentrations together with inadequate intake of vitamins and pro-oxidants can induce the development of cardiomyopathy, significant damage to the heart muscle, which makes these animals prone to heart attack, however, within all the mammalian species, the pygmy sperm whale (*Kogia breviceps*) is the most vulnerable to suffer from this type of disease (Bryan et al., 2017). In general, toxic levels of Se have been shown to cause deformities not only in this group of animals, but also in birds and fish. In addition, concentrations can be transferred from parents to progenitors by two routes: by biological mechanisms (during embryonic development) and by feeding habits (Se-rich prey) (Johnson et al., 2020). Although it was not the main objective of this study, in Table S2 we list some taxonomic groups (fish, marine mammals and birds) where metal(loids) contamination compromises their conservation.

Conclusions

We investigated drivers of biomagnification of Hg, As and Se in aquatic food webs. It is evident that the biomagnification potential of Hg is higher than that of As and Se. The latter two do not biomagnify in the same way as Hg. The results suggest that the biomagnification of these elements does not only depend on the level of concentration accumulated by the organisms, because the biology together with the ecology and physiology of the organisms can influence the biomagnification process. Furthermore, the complexity of the trophic structure and some environmental factors such as latitude and physicochemical characteristics of the waters (e.g. total phosphorus, oxygen, pH and total nitrogen) may also play an important role in the biomagnification of Hg in particular.

In general, it would be necessary to determine the strictly biological and physiological factors that lead to the biomagnification of these elements, since the influence of these factors seems not to be fully documented, especially for As and Se. There also seems to be a deficit of information revealing the possible incidence of environmental temporalities on the increase of As and Se concentrations through food webs.

From the biological point of view, the factors that should have priority are the influence of the growth rate of the different species and the influence of age, exposure periods and the metabolic processes of a given species on the modulation of As and Se concentrations, especially. These ideas are identified to the extent that it may be necessary to update and adjust the data we have in this regard and to generate broader and more reliable predictions of trophic rates of increase based on these variables. Finally, we suggest that biomagnification be incorporated into environmental management policies, mainly in risk assessment, monitoring and environmental protection programs.

Chapter 1

Supplementary Data

Table S1. Some studies on biomagnification of Hg, As and Se in food webs.

Metal(loids)	Environment	Country	Food web*	Driver	Reference
Hg	Tropical lake	Africa	b	Biomagnification is associated with the ecological dynamics and trophic level of organisms	Campbell et al., 2008
	Lake	Argentina	a	Concentrations are associated with food intake and size of organisms	Arcagni et al., 2013
	Subtropical lake	China	d	In long food chains, high stored concentrations can occur in superior predators in spite of the low rate of biomagnification experienced by subtropical deposits	Razavi et al., 2014
	River (neo tropic)	Venezuela	d	Concentrations increase depending on body mass	Kwon et al., 2012
	Artic	Canadá	e	Concentrations are influenced by the high productivity of the ecosystem	Clayden et al., 2015
	Tropical ocean	Brazil	f	Biomagnification patterns are evidenced in function of the increase in the trophic	Lemos-Bisi et al., 2012

				level, the feeding habit and the ecology of the species	
	Tropical marshes	Colombia	a	The increase in concentrations is linked to the trophic level and the state of anthropization of the ecosystem	Marrugo-Negrete et al., 2018
	Lake	USA	c	The concentrations were influenced by edaphic, physicochemical factors, eating habits and level of anthropic disturbance	Omara et al., 2015
	Estuary	Portugal	g	The biomagnification patterns were related to the trophic level. However, the age of the fish was the most important variable in explaining the increase in concentrations in the food web	Coelho et al., 2013
	Sea	Norway	i	There is a significant increase in concentrations mainly in seabirds related to the trophic level, but without any relationship between species	Jaeger et a., 2009
As	Estuary	Australia	c	Signs of biomagnification were evidenced. However, the total concentrations are similar to those present at unpolluted sites	Barwick & Maher, 2003

Sea	Canadá	j	It did not show a relationship with the trophic position, however, in the case of seabirds it showed evidence of important biomagnification	Campbell et al., 2005
Sea	Japan	h	The concentrations increased between rows of pelagic organisms, an increase that is linked to the soluble lipids in these organisms	Hayase et al., 2010
River	Vietnam	c	The concentrations differ between crustaceans and fish, a difference possibly attributed to the accumulation and detoxification capacity of the organisms	Ikemoto et al., 2008
Estuary	Australia	k	The concentrations were significantly higher in birds that were fed lower on the food chain	Einoder et al., 2018
Mangrove	Vietnam	l	Arsenobetaine is the most predominant As species in water. Biomagnification patterns are significant in the food web due to the presence of soluble lipids in organisms	Tu et al., 2011

Se	Lake	Argentina	a	In trophic chains composed of plankton and planktivorous fish, molar portions greater than 1 µmol are evidenced, mainly in fish	Arcagnic et al., 2013
	River	Vietnam	c	Concentrations increase depending on the trophic level between fish communities, however, there are differences in magnification profiles between species linked to detoxification mechanisms of some species	Ikemoto et al., 2008
	Estuary	Australia	c	High concentrations are observed in carnivorous fish related to eating habits	Barwinck & Maher, 2003

***Food web:** a=fish and plankton, b=fish-plankton-invertebrates and debris, c=fish-plankton and invertebrates, d=fish community, e=invertebrates, fish and seabirds, f= seston, fish, marine mammals and invertebrates, g= algae-fish-invertebrates and seagrasses, h= invertebrates, plankton and fish, i=fish, seabirds and plankton, j=invertebrates, fish, seabirds, marine mammals and algae, k=seabirds and sea fish, l=fish and macroinvertebrates.

Table S2. Overall conservation status of some species (fish, birds and marine mammals) reported in the reviewed studies.

Organisms	Common name	Scientific name	Country	*Category (IUCN)	Reference
Fish	Yellow fin bream	*Acanthopagrus australis*	Australia	LC	Barwick & Maher, 2003
	Eastern gambusia	*Gambusia holbrooki*		LC	Jasonsmith et al., 2008
	Bonylip barb	*Osteochilus microcephalus*	Vietnam	LC	Ikemoto et al., 2008
	Bigeye lates	*Lates mariae*	África	VU	Campbell et al., 2008
	Electric eel	*Malapterurus electricus*		LC	
	Walleye	*Sander vitreus*	Canadá	LC	Zhang et al., 2012
	Golden carp	*Carassius auratus*	Colombia	LC	Marrugo-Negrete et al., 2018
	Catfish	*Clarias gariepinus*		LC	Marrugo-Negrete et al., 2008
	Barbitetra	*Curimata mivartii*		NT	
	Shovelnose guitarfish	*Pseudobatos productus*	México	NT	Murillo-Cisneros et al., 2019
	Atlantic cod	*Gadus morhua*	Canadá	VU	Harding et al., 2018
	Red seabream	*Pagrus major*	Korea	LC	Kim et al., 2012
	Small puyen	*Galaxias maculatus*	Argentina	LC	Arcagni et al., 2017
	Bloater	*Coregonus hoyi*	EE UU	VU	Omara et al., 2015
	Shortjaw cisco	*Coregonus zenithicus*		VU	

	Argentine anchovy	*Engraulis anchoita*	Brazil	NT	Lemos-Bisi et al., 2012; Martins et al., 2020
	Banded croaker	*Paralonchurus brasiliensis*		LC	
	Lingua-de-mulata	*Symphurus tesselatus*		LC	
	Guitarfish	*Pseudobatos horkelii*	Brazil	CR	Martins et al., 2020
Birds	Long-tailed duck	*Clangula hyemalis*	Canadá	VU	Clayden et al., 2015
	Blacklegged kittiwake	*Rissa tridactyla*	Canadá	VU	Campbell et al., 2005
	Black guillemot	*Ceppus grylle*		LC	
	Ivory gull	*Pagophilia eburnea*		LC	
	Gull	*Larus hyperboreus*	Norway	LC	Jaeger et al., 2009
	Great tists	*Parus major*	Belgium	LC	Geens et al., 2010
	Ringed seals	*Phoca hispida*		LC	
Marine mammals	Walrus	*Odobenus rosmarus*		VU	
	Fin whales	*Balaenoptera physalus*		VU	Harding et al., 2018
	Minke whales	*Balaenoptera acutorostrata*		LC	

Sperm whale	*Physeter macrocephalus*	VU	Savery et al., 2013

*Category IUCN: International Union for Conservation of Nature: CR= critically endangered, LC = least concern, VU = vulnerable, EN= endangered, NT= near threatened. Categories are assigned according to information reported in the Red List database (https://www.iucnredlist.org/search?query=Phoca%20hispida&searchType=species)

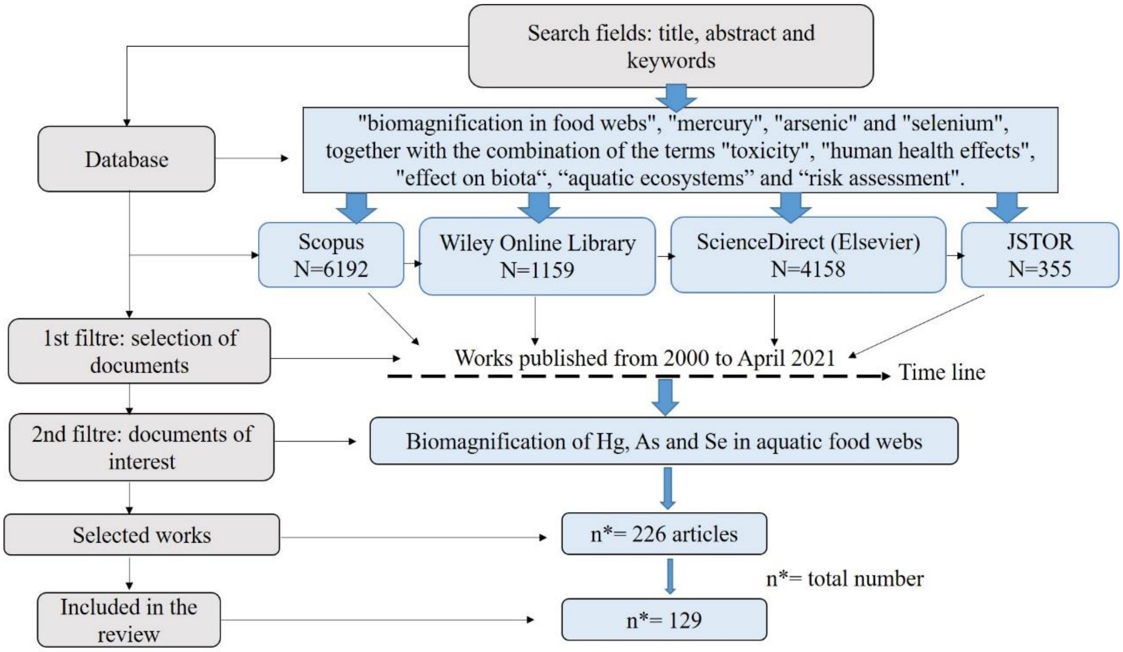

Figure S1. Proceedings and criteria considered for the search and selection of papers on biomagnification of Hg, As and Se in aquatic food webs.

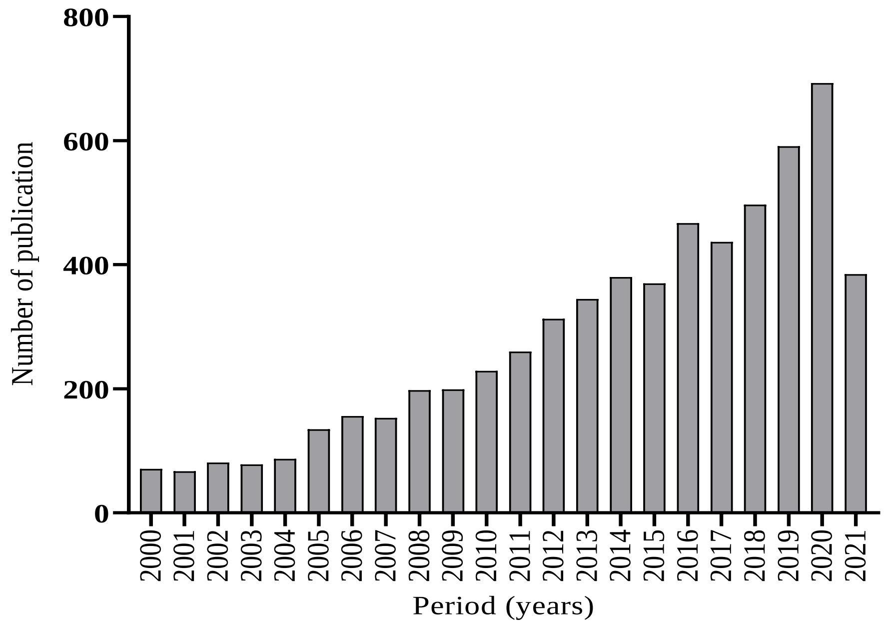

Figure S2. Scientific production on biomagnificación of metal(loids) in aquatic systems from 2000 to April 2021. Source: Scopus (https://www.scopus.com)

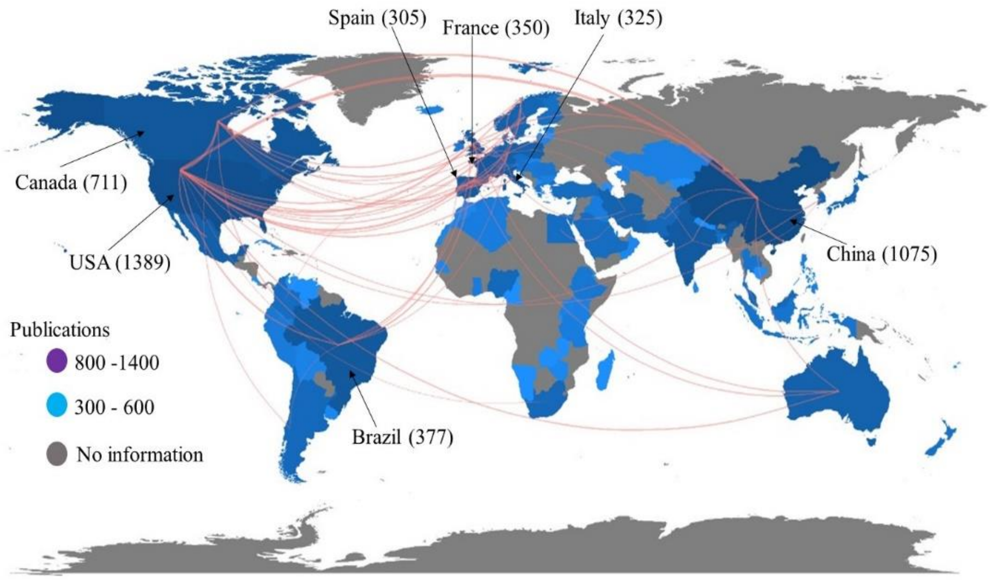

Figure S3. Countries with the highest scientific production on biomagnification of metal(loids) from 2000 to April 2021. The gray lines conform the network of collaboration between countries. Source: Scopus (https://www.scopus.com)

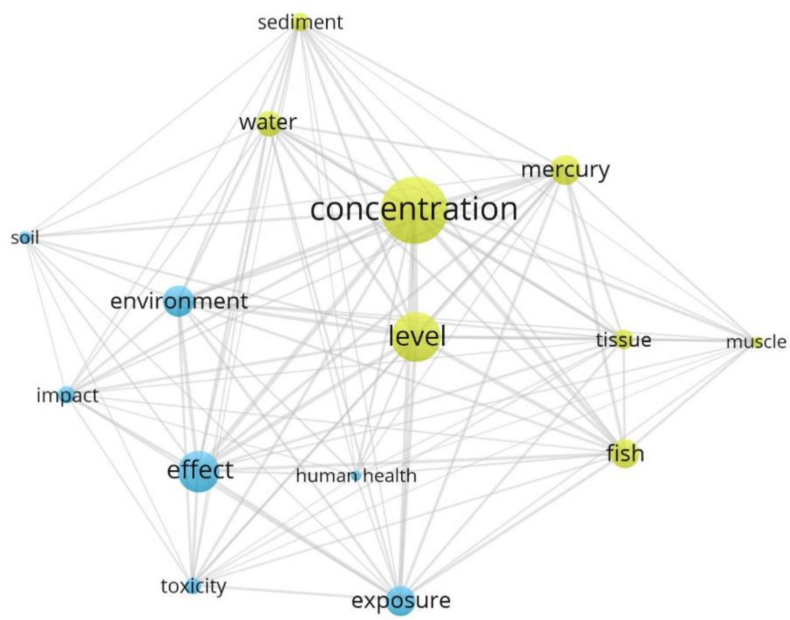

Figure S4. Co-occurrence of keywords most frequently used in studies of metal(loids) biomagnification in aquatic systems. The blue color encloses the oldest words within the set and the yellow color encloses the emerging words within the research. Source: Scopus (https://www.scopus.com)

Chapter 2. Toxic metal(loids) levels in the aquatic environment and nuclear alterations in fish in a tropical river impacted by gold mining

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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. In this study, we quantified the concentrations of mercury (Hg), methylmercury (MeHg) and arsenic (As) in aquatic environmental matrices, and explored for the first time nuclear degeneracies 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/kg) and for human consumption (500 µg/kg); while As concentrations were below the international standard (1,000 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, the T-Hg concentrations exceeded the threshold effect value of 12 ng/L. On the contrary, As concentrations for the same matrix were below the threshold of 10,000 ng/L as established by

USEPA. On the contrary, 33% of the sediments exceeded the quality standard of 0.2 µg/g for Hg. According to the ACP, sediments and water could be an important source of contamination in the Atrato River. 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.

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 the main receptors of contaminants, as well as good indicators of aquatic 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 & 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 & 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 conserve biodiversity (Marrugo-Negrete et al., 2018; Reid et al., 2020; UNEP, 2017; Vicari et al., 2012; 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.

Materials and methods

Study area

The Atrato basin is located in the Pacific region of Colombia in the department of Chocó. According to the most recent census of the National Statistics Department (DANE), 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 Figure 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). Figure 2 shows a visual overview of the sampling stations.

The basin has a length of 750 km, a surface area of 35, 700 km^2 and an average annual flow of 4,137m^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 & 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 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).

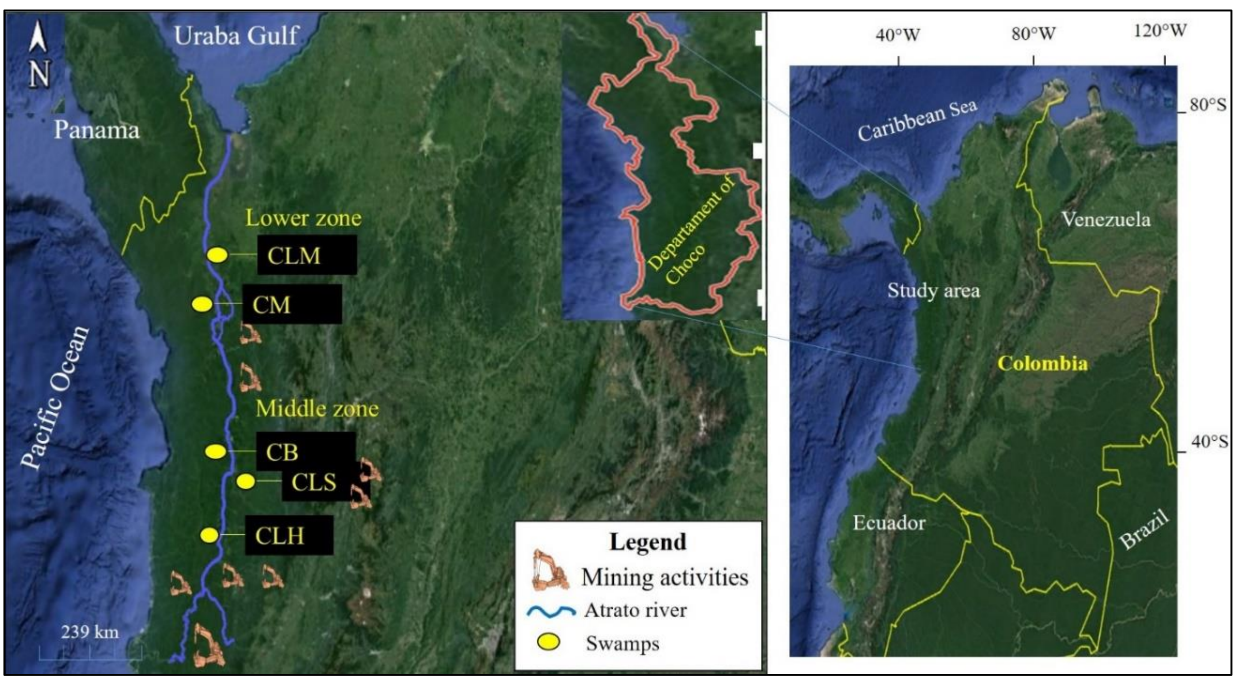

Figure 1. Geographical location of the study area and sampling stations (swamps) in the Atrato River basin, Colombia.

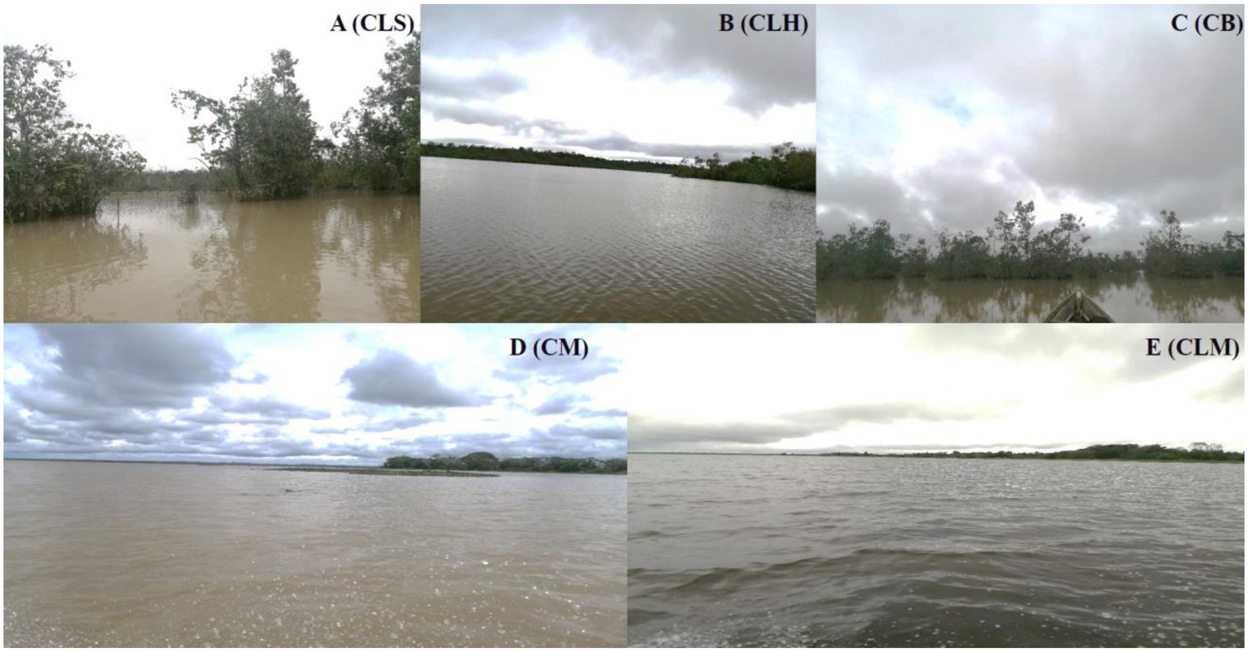

Figure 2. Visual panorama of the sampling stations in the Atrato River basin. The images are placed according to the location of the bogs along the basin. Source: The author

Collection of fish, water and sediment

A total of 205 fish were collected, grouped into 14 genus and 15 species. In each site, fish were collected randomly in the dry season. The number of fish per station were distributed as follows: CLH (n=40), CLS (n=20), CB (n=60), CLM (n=36) and CM (n=49). 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 to 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 (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.

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 iCE^{TM} 3500 - Waltham, MA, USA) after digestion with dilute $KMnO_4$-$K_2S_2O_8$ 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 iCE^{TM} 3500 - Waltham, MA, USA), with prior microwave-assisted acid digestion according to EPA method 3051A for sediments (USEPA, 2007), EPA 3015A 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 Canadá (NRCC) DORM-2 dogfish muscle standard. The percent recovery of T-Hg was 99.6 ± 0.2% (n=3), and the detection limit (0.003 µg/g) 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 detection limit of 16 µg/kg. For quality control for As, calibration curves were constructed with R^2 > 0.998, with a detection limit 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). All T-Hg, MeHg and As concentrations 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 K_d 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 & Allison, 2005).

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 three hours before spreading. The slides together with the blood sample were fixed with 99% methanol for one minute and allowed to air dry for ten minutes. After this time the slides were stained with Giemsa stain for ten minutes. The slides were then washed with distilled water and left to dry at room temperature for 12 hours (Hussain et al., 2018; Melo-Silva et al., 2018; Obiakor et al., 2014).

In the laboratory nuclear abnormality counting was performed on 2,000 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, Gaviria-Angulo, 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). Figure 3 shows a summary of the procedure performed for the micronucleus test.

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 ($K=100\ W/L^3$). A value of $K \geq 1$ 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 & Abdul, 2020; Froese, 2006).

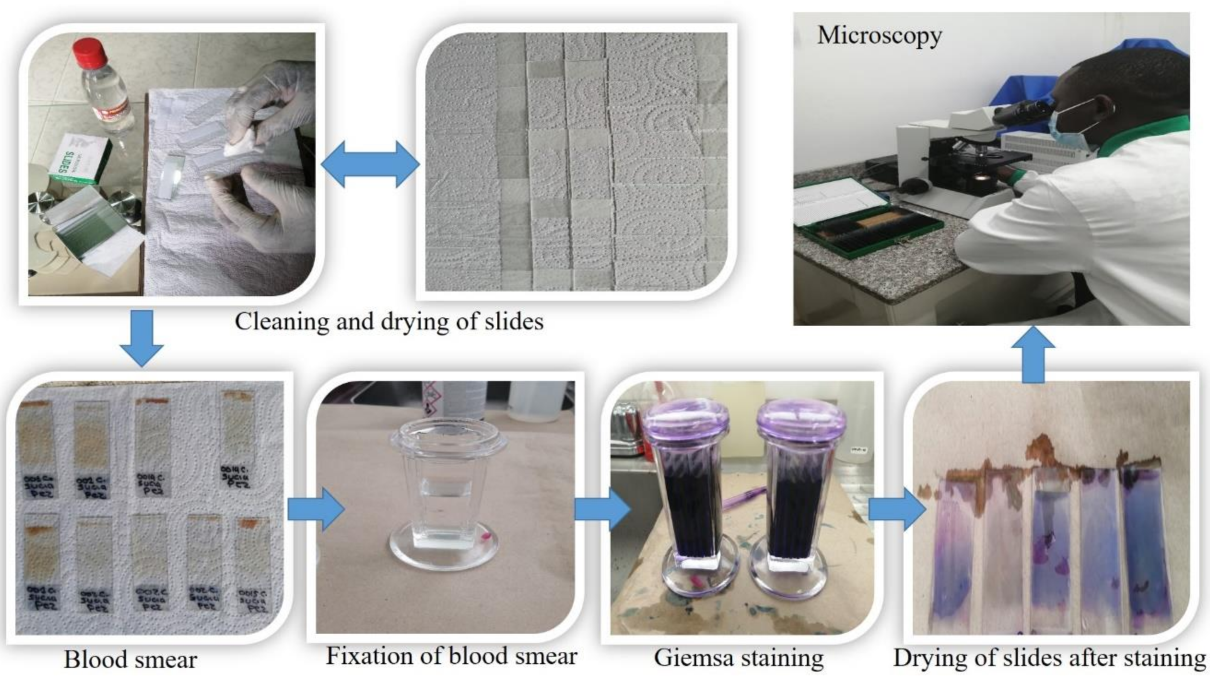

Figure 3. Summary of technique used for micronucleus test.

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, Gaviria-Angulo, 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.

Results

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%).

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 (Figure 3) 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 individuals) were between 6.1 and 199.7 µg/kg.

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±2.12 (21.0-24.0)	57.5±3.53 (55.0-60.0)
Cathorops melanopus	Bagre blanco	1	C	4.3	357.0	43	643
Pseudopimelodus schultzi	Bagre sapo	2	C	3.7	516.5±7.04 (511.5-521.5)	46.0±4.22 (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±1.21 (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±5.68 (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±1.93 (22.0-34.0)	195.0±52.9 (117.0-466.0)
Colossoma macropomum	Cachama	1	O	2.0	668.12	94	15.515
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	6.10	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).

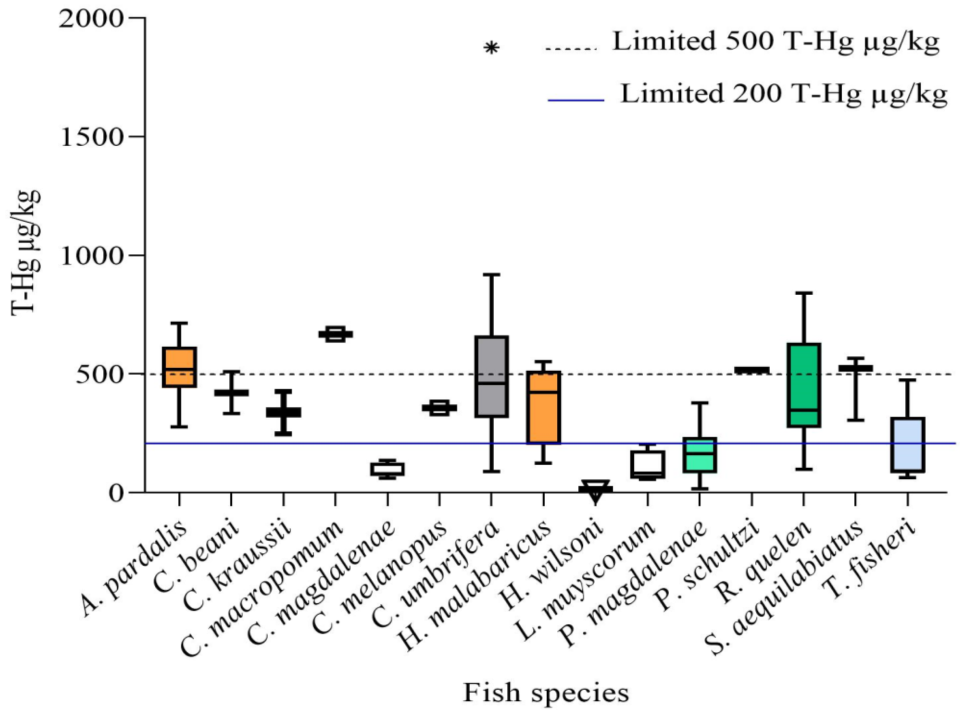

Figure 3. T-Hg concentrations in fish collected in swamps of the Atrato River basin, Colombia. The dashed line refers to the thresholds recommended by FAO/WHO.

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 (Figure 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% (Figure 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.

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±3.903	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	547.64	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	5.25	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

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 1,000 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 ($p > 0.05$).

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 1594 de 1984). 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 Figure 4. Overall, metal(loids) concentrations in sediment ranged from 56.8-287.6 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. 33.3% of the sediment samples (Figure 4a) 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, concentrations ranged from 5.0 to 331.0 T-Hg ng/L and 0.5-4.5 As ng/L with an average of 154.7±116.5 T-Hg ng/L and 2.1±1.1 As ng/L (Figure 4b). 60% of the water samples collected exceeded the 2,000 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±52.0 ng/L, $p < 0.05$).

Principal component analysis

As shown in Figure 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.

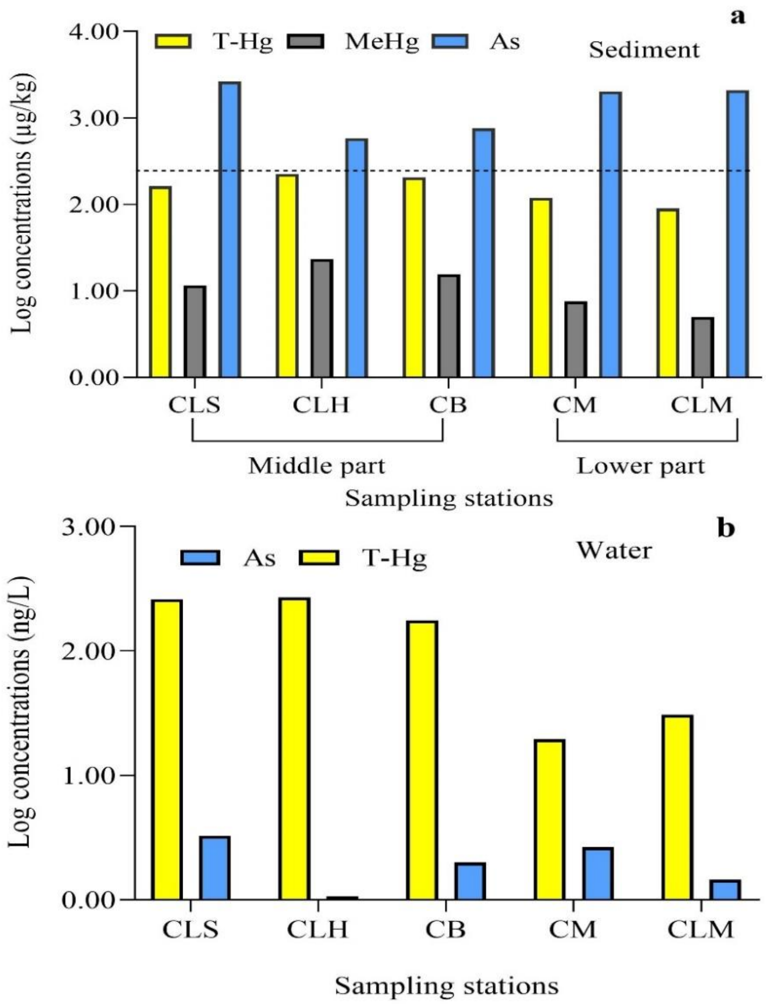

Figure 4. Distribution of metal concentrations in water and sediment samples collected in different stations in the Atrato River basin, Colombia. The dashed line indicates the quality value of 200 µg/kg USEPA, 2000).

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 ≥ 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 frequency 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, Figure 5). No alterations were evident among the total (10) fish examined in the lower part of the basin. Spearman correlation between T-Hg (r^2 = 0.10, p > 0.05), As (r^2 = -0.21, p > 0.05) and MN frequency showed no significant association between the variables. However, it is striking that fish collected in the middle waters, where total Hg contamination was higher, showed nuclear degeneration compared to fish collected in low waters. 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.

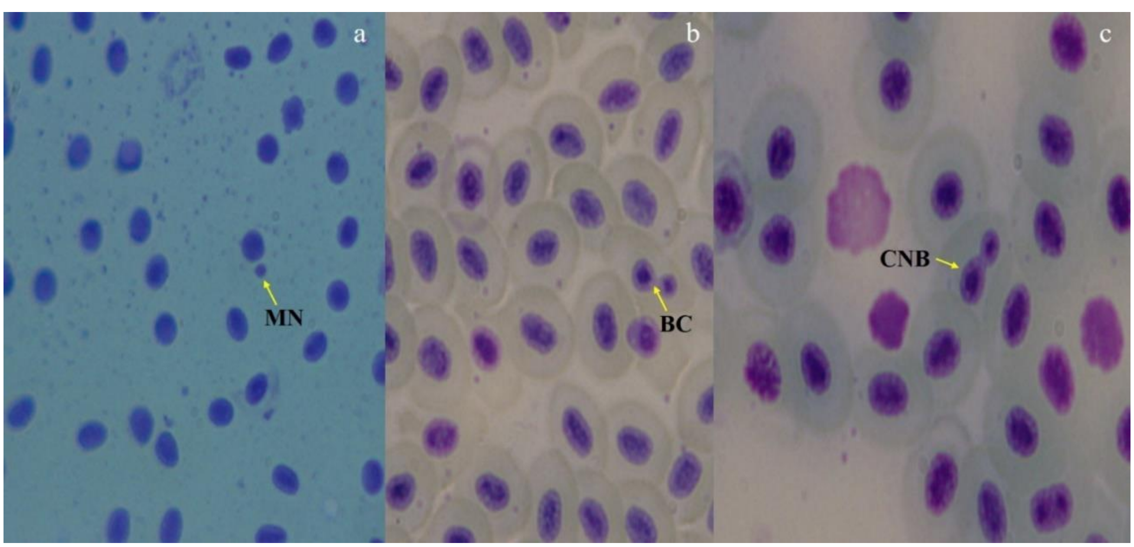

Figure 5. Microscopic capture of micronucleus and other nuclear abnormalities in *P. magdalenae* species, a= micronucleated cell (MN), b= binucleated cells (BC) and c= cells with nuclear buds (CNB).

Discussion

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 S5.

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 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).

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 1,732 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).

Distribution coefficients in sediment and water

The K_d 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 K_d of 67 and 79 for T-Hg and As, respectively (Figure 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 & Allison, 2005), as the mobilization, distribution and accumulation of metals may be subject to physicochemical and biogeochemical conditions of the particular environment (Cui & Jing, 2019; Mukherjee et al., 2014; Paschoalini & Bazzoli, 2021). In general, K_d values for Hg when logarithmically transformed in aquatic environments (e.g. lakes, rivers and streams) show an average variation between 4.2-6.9 (suspended material/water), 3.8-6.0 (sediment/water) and 2.2-5.8 (soil/water) globally (Allison & 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 & Rudd, 1990). In this sense, K_d values can be affected by physicochemical factors including pH (Allison & Allison, 2005).

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 ≥ 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 & 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 ≥ 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; Silva 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).

Although there was no significant relationship between %MN and Hg and As concentrations, it is noteworthy that fish collected upstream showed greater nuclear changes in areas with higher Hg concentrations. This suspicion suggests further research to help decipher more clearly the toxic agents responsible for genetic degeneration in fish in the Atrato river basin (Cruz-Esquivel et al., 2023; Vieira et al., 2022).

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.

Chapter 2

Supplementary Data

Table S1. Composition and ecological features of fish collected in the five stations in the Atrato River basin, Colombia.

Scientific name	Common name	n	Species %	Trophic role	Life form	Status*
Caquetaia umbrifera (Meek & Hildebrand. 1913)	Mojarra negra	12	5.9%	Carnivore	Benthopelagic	
Hoplias malabaricus (Bloch. 1794)	Quicharo	11	5.4%	Carnivore	Benthopelagic	
Sternopygus aequilabiatus (Humboldt. 1805)	Beringo	3	1.5%	Carnivore	Benthopelagic	
Cathorops melanopus (Günther. 1864)	Bagre blanco	1	0.5%	Carnivore	Benthopelagic	
Pseudopimelodus schultzi (Dahl. 1955)	Bagre sapo	2	1.0%	Carnivore	Benthopelagic	
Ctenolucius beani (Fowler. 1907)	Agujeta	2	1.0%	Carnivore	Pelagic	
Prochilodus magdalenae (Steindachner. 1879)	Bocachico	134	65.4%	Detritivore	Benthopelagic	
Cyphocharax magdalenae (Steindachner. 1878)	Jojorro	7	3.4%	Detritivore	Benthopelagic	
Hemiancistrus wilsoni (Eigenmann. 1918)	Guacuco corroma	1	0.5%	Detritivore	Bentonic	
Colossoma macropomum (Cuvier. 1816)	Cachama	1	0.5%	Omnivore	Benthopelagic	
Leporinus muyscorum (Steindachner. 1900)	Denton	4	2.0%	Omnivore	Benthopelagic	
Rhamdia quelen (Quoy & Gaimard. 1824)	Barbudo	7	3.4%	Omnivore/carnivore	Benthopelagic	
Caquetaia kraussii (Steindachner. 1878)	Mojarra amarilla	2	1.0%	Omnivore/carnivore	Benthopelagic	
Trachelyopterus fisheri (Eigenmann. 1916)	Caga	9	4.4%	Piscivore	Pelagic	
Ageneiosus pardalis (Lütken. 1874)	Doncella	9	4.4%	Piscivore	Pelagic	
Total		205	100%			

Status: gray= least concern, green= not evaluated and red= vulnerable.

Table S2. Comparison of T-Hg concentrations (median ± standard deviation, in µg/kg) between the five stations according to location in the Atrato River basin.

Stations	**Part basin**	**T-Hg**	**Range**	
CLH	Middle	178.5±130.2	40.0-551.7	
CLS	Middle	112.5±223.8	6.1-714.1	
CB	Middle	**234.9***±151.4	42.0-919.8	
CLM	Lower	101.8±176.7	15.6-668.1	
CM	Lower	158.4±298.5	28.9-1876	
Dunn's multiple comparisons test				
	p value	Mean rank diference	Kruskal-Wallis statistic	
CLS vs. CLH	>0.9999	-12.3	K= 13.8. $p < 0.05$	
CLS vs. CB	0.7277	-27.48		
CLS vs. CLM	>0.9999	17.78		
CLS vs. CM	>0.9999	-7.02		
CLH vs. CB	>0.9999	-15.18		
CLH vs. CLM	0.2731	30.08		
CLH vs. CM	>0.9999	5.28		
CB vs. CLM	0.003	45.26		
CB vs. CM	0.7322	20.46		
CLM vs. CM	0.5687	-24.8		
Concentrations (T-Hg) according to account location				
Middle part*	Range	Lower part	Range	Mann-Whitney U
209.0±158.6	6.1-919.8	126.1±256.7	15.6-1876	U= 3039, $p < 0.05$

*=The medians highlighted in bold were statistically different.

Table S3. Arsenic concentrations (As, median ± standard deviation, range, in µg/kg) in fish collected in stations in the Atrato River basin, Colombia.

Species	n	Trophic level	As µg/kg	Range
Ctenolucius beani	2	4.0	167.5±62.1	123.5-211.4
Cathorops melanopus	1	4.3	30.0	-
Pseudopimelodus schultzi	2	3.7	30.0.5±0.0	30.0-30.0
Rhamdia quelen	7	3.9	60.4±24.1	15.0-68.9
Sternopygus aequilabiatus	3	3.2	30.0±26.3	30.0-75.6
Prochilodus magdalenae	134	2.2	30.8±26.2	15.5-200.8
Colossoma macropomum	1	2.0	30.0	-
Trachelyopterus fisheri	9	3.5	37.2±18.2	15.0-68.0
Leporinus muyscorum	4	2.2	52.0±23.5	30.0-81.1
Ageneiosus pardalis	9	3.8	30.0±96.0	15.0-263.8
Hemiancistrus wilsoni	1	2.2	38.18	-
Cyphocharax magdalenae	7	2.0	30.0±14.5	15.0-62.9
Caquetaia kraussii	12	3.4	40.8±36.5	15.0-66.7
Caquetaia umbrifera	2	3.8	30.0±14.0	15.0-71.6
Hoplias malabaricus	11	4.5	30.0±59.4	15.0-187.6
Total	205		30.0±36.8	15.4-263.8

Table S4. Descriptive statistics (mean ± standard deviation) of the cumulative frequency of nuclear anomalies: micronucleus (MN), cells with nuclear buds (CNB) and binucleated cells (BC) found in fish collected in the stations investigated in the Atrato River, Colombia. The data correspond only to fish evaluated in mid-water.

Species	**n**	**MN**	**CNB**	**BC**
		X̄±SD+Range	**X̄±SD+Range**	**X̄±SD+Range**
P. magdalenae	16	1.6±2.5 (0.0-9.0)	3.7±5.4 (0.0-21.0)	1.6±2.3 (0.0-9.0)
L. muyscorum	4	1	-	-
C. kraussii	2	0.5±0.7 (0.0-1.0)	1.0±0.0 (1.0-1.0)	0.5±0.7 (0.0-1.0)
R. quelen	7	-	1	2
H. wilsoni	1	-	-	-
C. beani	3	-	-	-
Total	33			

(-) not detected

Table S5. 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	Chocó	Colombia	678.5	Salazar-Camacho et al., 2021
	23	Atrato River	Chocó	Colombia	950.0	Palacios-Torres et al., 2018
Ctenolucius beani	28	Atrato River	Chocó	Colombia	270.9	Salazar-Camacho et al., 2021
Caquetaia kraussii	85	Atrato River	Chocó	Colombia	218.0	Salazar-Camacho et al., 2021
	44	Atrato River	Chocó	Colombia	240.0	Palacios-Torres et al., 2018
	2	Río Las marías	-	Venezuela	1020.0	Kwon et al., 2012
Cyphocharax magdalenae	18	Atrato River	Chocó	Colombia	32.0	Salazar-Camacho et al., 2021

	23	Atrato River	Chocó	Colombia	60.0	Palacios-Torres et al., 2018
Pseudopimelodus schultzi	22	Atrato River	Chocó	Colombia	432.7	Salazar-Camacho et al., 2021
Leporinus muyscorum	42	Atrato River	Chocó	Colombia	116.7	Salazar-Camacho et al., 2021
Prochilodus magdalenae	135	Atrato River	Chocó	Colombia	93.1	Salazar-Camacho et al., 2021
	26	Atrato River	Chocó	Colombia	140.0	Palacios-Torres et al., 2018
Rhamdia quelen	76	Atrato River	Chocó	Colombia	145.8	Salazar-Camacho et al., 2021
	2	Río Las marías	-	Venezuela	370,0	Kwon et al., 2012
Hoplias malabaricus	87	Atrato River	Chocó	Colombia	401.4	Salazar-Camacho et al., 2021
	46	Atrato River	Chocó	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	Kwon et al., 2012
Ageneiosus pardalis	9	Swamp, Atrato River	Chocó	Colombia	517.9	This study
Ctenolucius beani	2	Swamp, Atrato River	Chocó	Colombia	420.7	This study
Caquetaia kraussii	2	Swamp, Atrato River	Chocó	Colombia	337.6	This study

Cyphocharax magdalenae	7	Swamp, Atrato River	Chocó	Colombia	78.3	This study
Pseudopimelodus schultzi	2	Swamp, Atrato River	Chocó	Colombia	516.5	This study
Leporinus muyscorum	4	Swamp, Atrato River	Chocó	Colombia	81.9	This study
Prochilodus magdalenae	134	Swamp, Atrato River	Chocó	Colombia	165.2	This study
Rhamdia quelen	7	Swamp, Atrato River	Chocó	Colombia	347.7	This study
Hoplias malabaricus	11	Swamp, Atrato River	Chocó	Colombia	422.7	This study

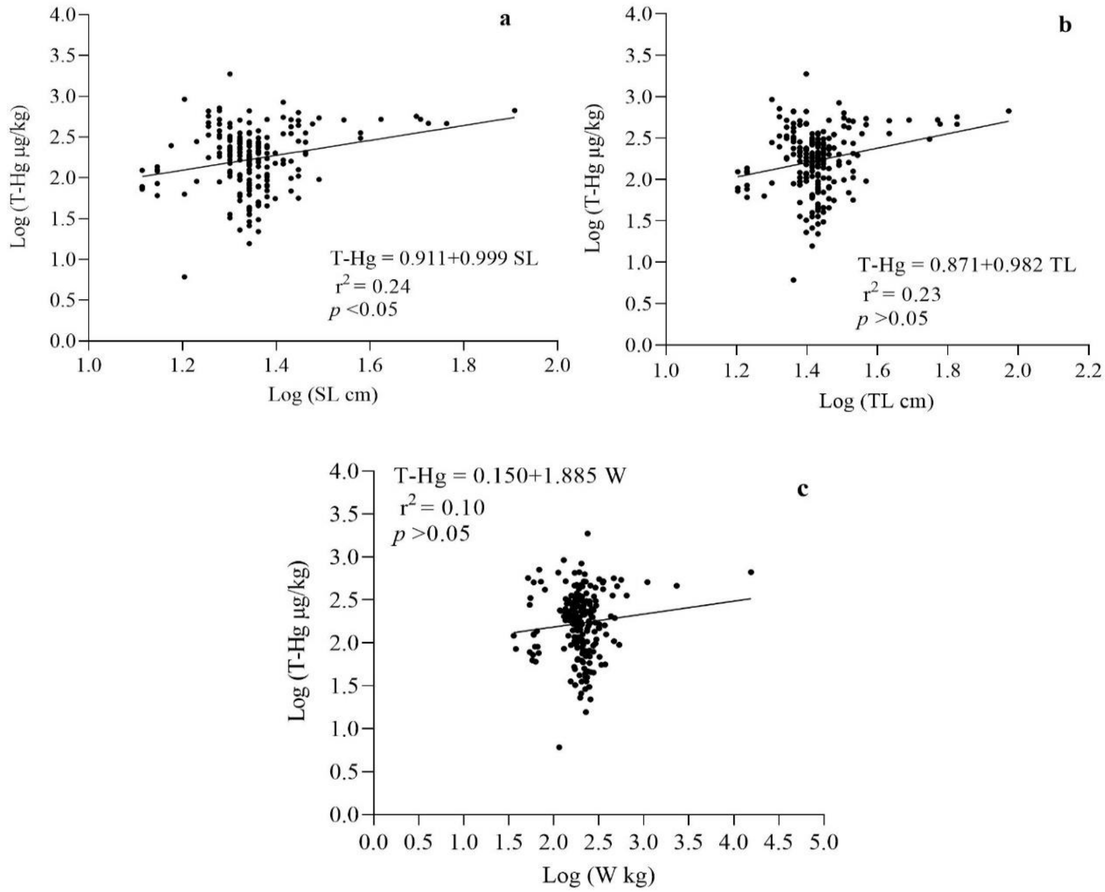

Figure S1. Relationship between T-Hg concentrations and biometric variables of fish (n =205) collected in the five stations in the Atrato River basin, Colombia, a=standard length-SL (cm) vs THg, b=total length-TL (cm) vs T-Hg and c=weight-W (kg) vs T-Hg.

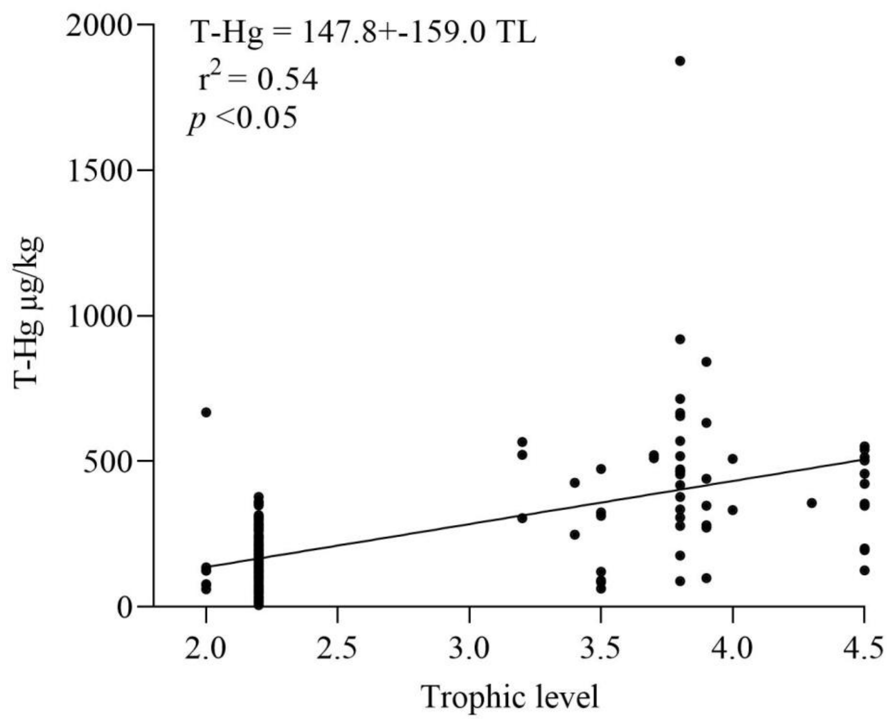

Figure S2. Spearman relationship between T-Hg concentrations and trophic level.

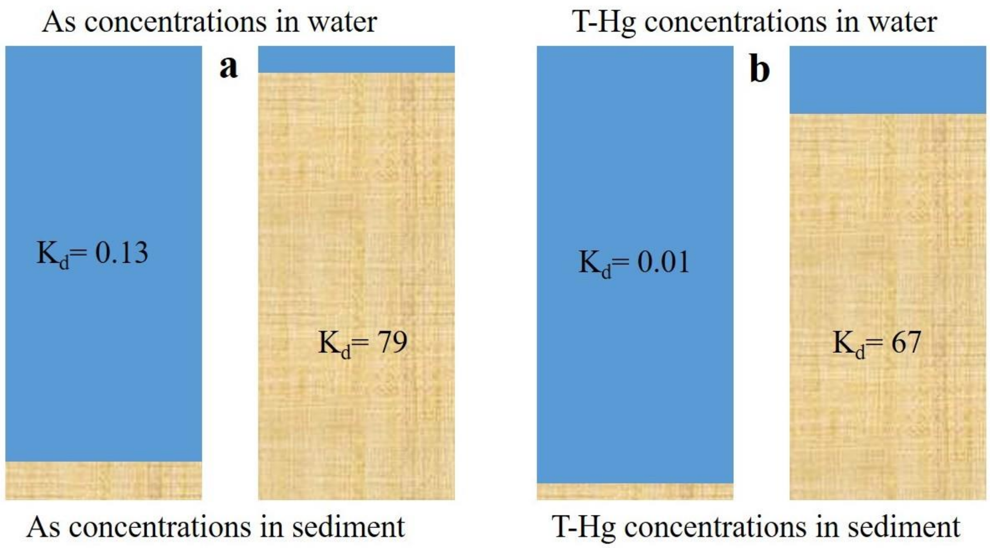

Figure S3. (a) distribution coefficient As of and (b) Hg in water and sediment in the five stations of the Atrato River basin, Colombia.

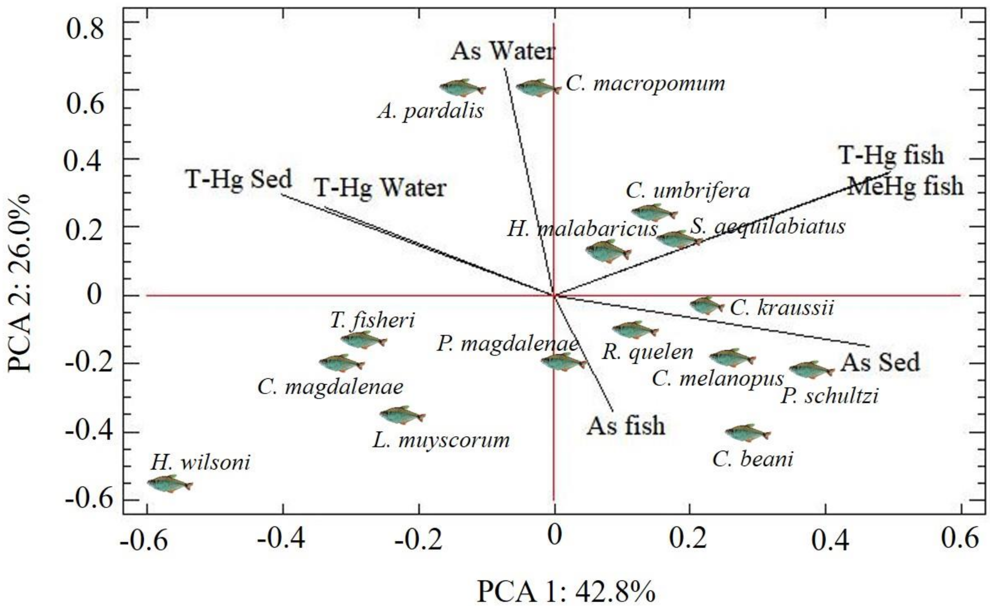

Figure S4. Biplot of the first two components of the principal component analysis (PCA), including concentrations of T-Hg, MeHg and As measured in water, sediments and fish collected in the five stations in the Atrato River basin, Colombia.

Chapter 3. Ecological and human health risk from exposure to contaminated sediments in a tropical river impacted by gold mining in Colombia

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Abstract

Despite being one of the most important tropical biomes in the world, the Atrato River basin has experienced a critical ecological deterioration due to gold mining, posing a significant threat to wildlife and human health. In this study, we measured the concentrations of mercury (Hg) and arsenic (As) in sediments at various swamps within the basin. Classical indices were employed to assess the associated ecological and human health risks linked to exposure to these elements. The concentrations of Hg and As in the sediments ranged between 0.09 to 0.23 mg/kg and 0.59 to 2.68 mg/kg, respectively. The highest Hg values were found at upstream stations impacted by gold mining activities. For As, the highest levels were found near river mouth (except for station B), where agricultural practices are taken place. The contamination factor (CF) indicated that most of the sediments exhibited moderate contamination levels of Hg and As, depending on the specific sampling area. Conversely, the pollution load index (PLI) suggested a contamination level ranging from basic to moderate for, with the exception of station B, which showed a progressive deterioration of the site. The geoaccumulation index (Igeo) indicated that the sediments were moderately contaminated with Hg, while showing signs of increasing contamination for As. According to the criteria for limiting effect concentrations (TEC), Hg concentrations exceeded the TEC at stations A and C, indicating a potential toxic risk to aquatic biota. A moderate potential ecological risk (PERI) was detected at downstream stations (D and E), and a high risk was detected at upstream stations (A, B and C). The hazard index (HI), used for non-carcinogenic risk assessment, suggested a risk of adverse effects on the population, particularly in children, with HI values exceeding 1. However, all lifetime cancer risk (TLCR) values fell within the acceptable range (1 x 10^{-6} to 1 x 10^{-4}), indicating a negligible risk. Oral ingestion and inhalation were identified as the two primary routes of concern. This study serves as a valuable reference for risk assessment regarding exposures to environmental matrices that may not pose an immediate risk to human health.

Keywords: Atrato River, mining, metal(loids), sediments, risk assessment.

Introduction

The contamination of rivers with mercury (Hg) and arsenic (As) is a significant environmental challenge that concerns researchers worldwide due to the detrimental effects these elements can have on wildlife and human health (Cobbina et al., 2015; Sigmund et al., 2023). The presence of these elements in the environment can stem from natural processes, such as atmospheric deposition, rock weathering, and erosion (Branfireun et al., 2020; Hanfi et al., 2020; Pang et al., 2022). However, human activities, particularly mining and agriculture, greatly influence the release and rise of concentrations of these elements in aquatic environments (Badrzadeh et al., 2022; Mohammadi et al., 2022).

Once metal(loids) are released into the aquatic environment, they become incorporated into the water and accumulate in sediments. They can then move up the food chain, affecting the health of both wildlife and humans (Ali & Khan, 2018a; Kusin et al., 2018; Magni et al., 2021). Extensive research has shown that both Hg and As can have diverse impacts on wildlife, ranging from reduced reproductive capacity to significant effects on the immune system, thereby posing a threat to biodiversity conservation (Kumari et al., 2017; Zheng et al., 2019). As is a metalloid that exhibits high toxicity and can cause kidney damage, induce the development of cancer, and affect the central nervous system. On the other hand, Hg, also a highly toxic metal, can cause similar effects, except for cancer, as it has been classified as non-carcinogenic (USEPA, 1989, 2004).

Many researchers concur that the primary routes of exposure to toxic metal(loids) in cases of sediment contamination are oral ingestion, inhalation, and dermal contact (Cobbina et al., 2015; Hoang et al., 2021). For instance, when individuals engage in swimming activities in rivers, there is a likelihood of incidental ingestion of small volumes of potentially contaminated water (Hoang et al., 2021; USEPA, 2023a). In addition, ingestion of fish contaminated with sediment-derived metal(loids), substantially increases the risk to human health (Calao-Ramos et al., 2021), since fish constitute the third major source of dietary protein consumed by humans after cereals and milk (Tacon & Metian, 2018).

Sediments play a role in trapping toxic metals that can subsequently release them into the water column, further increasing the potential for direct and indirect contact with hazardous contaminants (Jia et al., 2021). As a result, this scenario creates a conducive environment for individuals to come into contact with these harmful substances (Ng et al., 2021; Şimşek et al., 2022; USEPA, 2023a).

In general, sediment contamination poses risks not only to wildlife but also to human health. Consequently, assessing sediment quality plays a key role in understanding the extent of contamination in aquatic ecosystems and enables the development of measures to protect aquatic biota and community health (Mohajane & Manjoro, 2022; Xiao et al., 2022).

In response, there is a global consensus that defines threshold effect concentration (TEC) and probable effect concentration (PEC) criteria for the assessment of sediment quality (MacDonald et al., 2000). These criteria serve as guidelines for evaluating the impact of sediment contamination. In addition, various indices such as the pollution load index (PLI), contamination factor (CF), and potential ecological risk index (PERI) have found extensive applicability in the field of environmental sciences for assessing the ecological risks associated with the presence of toxic metal(loids) in sediments. These indices have gained popularity due to their ability to provide rapid, valuable, and reliable information for decision-making processes (Abdullah et al., 2015; Maanan et al., 2015; K. S. Vieira et al., 2021). Regarding human health risk assessment, the total hazard index (HI) and the lifetime cancer risk index (LCR) are among the most commonly used approaches to evaluate non-carcinogenic and carcinogenic risks, respectively (Canpolat et al., 2022; Dong et al., 2023; Price, 2023).

The Atrato River is one of the most important tropical ecosystems in Colombia and globally, but it faces significant impacts from gold mining and agricultural expansion (Palomino-Ángel et al., 2019). Previous studies conducted in the region have focused on assessing the risks associated with toxic metal(loids) concentrations in various components, including human populations (Palacios-Torres et al., 2018), water (Marrugo-Madrid et al., 2022), plants (Marrugo-Negrete et al., 2016, 2017), sediments (Palacios-Torres et al., 2020) and fish (Salazar-Camacho et al., 2021, 2022).

Additionally, recent studies have investigated the concentrations of toxic metal(loids) in fruits of gastronomic importance (Caicedo-Rivas et al., 2023), and the evaluation of genotoxic effects in fish (Córdoba-Tovar et al., 2023). However, despite these advances in research, our comprehensive understanding of the environmental contamination caused by human activities in the area remains limited. The objective of this study is to evaluate the ecological risks associated with Hg and As concentrations in sediments of the Atrato River, integrating this assessment with the risks to human health. By examining both ecological and human health aspects, this work aims to provide a more holistic understanding of the environmental contamination induced by anthropic sources in the region.

Materials and methods

Study area

The Atrato River is one of the main watersheds in Colombia. It is located in the department of Chocó, one of the richest territories in biodiversity located in western Colombia (Figure 1). The basin is 750 km long and covers an area of 35,700 km^2. It also has one of the highest average annual flows (4,137 m^3/s) in the world.

The annual rainfall reported for the basin ranges between 5,000 and 12,000 mm/year (Palomino-Ángel et al., 2019) with maximum temperatures ranging between 27 and 31 °C, and minimum temperatures between 22 and 23 °C (Asprilla et al., 2018). These characteristics have partly contributed to the formation of ecologically important ecosystems (e.g. tropical rainforests and wetlands), which together occupy 88% of the basin's surface area (Palomino-Ángel et al., 2019; Velásquez & Poveda, 2019). The Atrato basin has an elongated synclinal shape with a depression with sedimentary consequences. Generally, the geological deposits are Quaternary terraces, beach sediments and alluvium (INGEOMINAS, 2003). According to more recent statistics from the National Administrative Department of Statistics (DANE, for its acronym in Spanish), the population settled over the Atrato basin is at least 664,000 inhabitants concentrated in greater proportion in the sub-region of Darien in Bajo Atrato (DANE, 2018). The economy is mainly based on fishing, mining and agriculture, with the latter two having the greatest impact on environmental changes in the Atrato River (Caicedo-Rivas et al., 2023; UNODC, 2016).

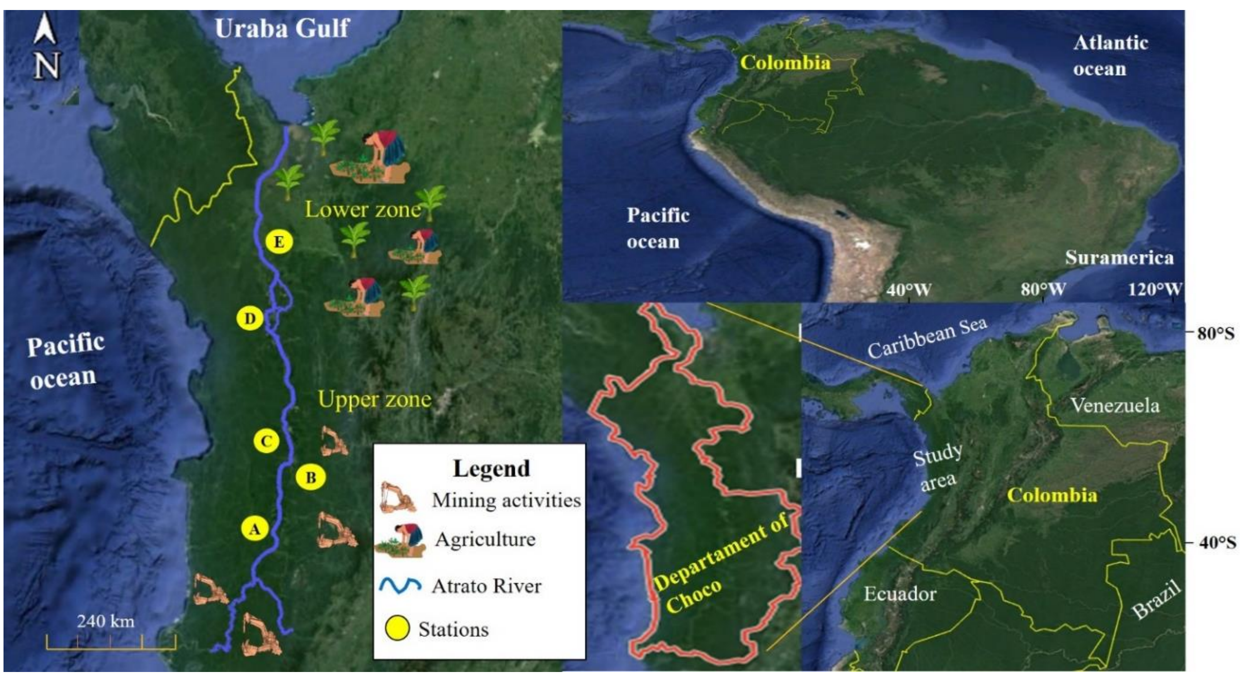

Figure 1. Map of the geographical location of the study area in Colombia and sediment sampling sites in the Atrato watershed. The sampling sites included swamp Honda (station A), swamp Sucia (station B), swamp Baudocito (station C), swamp Montaño (station D), and swamp los Medios (station E).

Collection of sediment

We collected sediments from two differenced swamps or stations in the Atrato River, the upper zone (including stations A, B and C) and the lower zone (including stations D and E), close to the river mouth. The upper zone is impacted by gold mining, whereas the lower zone is impacted by agrochemicals due to agriculture and soil erosion (Blanco-Libreros, 2016). Therefore, between July and August 2021, sediment samples were collected in five swamps from upstream to downstream (A to E), according to A (swamp Honda); B (swamp la Sucia); C (swamp Baudocito), D (swamp Montaño) and E (swamp los Medios). The stations were prioritized based on the community's perception of their significance in their daily activities. Five sediment subsamples (0.5 kg) were collected at each station with the aid of a boat-launched Ekman dredge (Figure 2) (Marrugo-Negrete et al., 2010; Palacios-Torres et al., 2018). The samples were taken at different cardinal points georeferenced with a GPS within a radius of 2m from the reference point. This procedure was repeated three times at each station at a distance of approximately 100 m in order to maximize coverage of the area. All the samples were mixed until obtaining a composite sample per station, for a total of three samples/station. The samples were stored in plastic bags, labeled and refrigerated until they were transferred to the laboratory (Gutiérrez-Mosquera et al., 2020; Jia et al., 2021).

Figure 2. Summary of sediment sample collection process. A (dragra release), B (sample preparation), C (sample storage).

Analysis of mercury and arsenic in sediment

For the analysis of Hg and As, a 0.5 g of sediment sample was digested with H_2SO_4/HNO_3 7:3 v/v and 5 % w/v $KMnO_4$ at a temperature of 100 °C for 1 h. Hg concentrations were determined by thermal decomposition, detected by atomic absorption spectrometry following method 7473 suggested by the United States Environmental Protection Agency-USEPA (USEPA, 1998). A direct Hg analyzer (DMA-80, Milestone Srl, Italy) with a detection limit of 0.026 mg/kg was used for this analysis (Gutiérrez-Mosquera et al., 2021; Marrugo-Negrete et al., 2021). The detection limit was calculated as three times the standard deviation of 10 blank measurements divided by the slope of the calibration curve (0.013 mg/kg) of fresh weight. The reference material (CRM008-050, Resource Technology Corporation) of 0.74 + 0.02 mg/kg (certified value 0.72 + 0.03 mg/kg) was used for quality control (Marrugo-Negrete et al., 2010).

Concentrations of As were analyzed by hydride generation atomic absorption spectroscopy (HGAAS, SM3114B) with prior microwave-assisted acid digestion according to EPA method 3051A (USEPA, 2007b), using a Thermo Elemental Solaar S4 spectrometer (Marrugo-Negrete et al., 2010). For As quality control, calibration curves ($R^2 > 0.998$) were constructed with a detection limit of 49.66 mg/kg (IAEA 405 reference material) (Marrugo-Negrete et al., 2021). Hg and As concentrations were expressed in mg/kg wet weight, and were analyzed in duplicate. All analyses were performed at the Laboratory of Toxicology and Environmental Management at the University of Cordoba, Colombia.

Risk assessment methods

The ecological risk was evaluated using different classic indices used by researchers in environmental sciences. These indices were pollution factor (CF), geoaccumulation index (Igeo), pollution load index (PLI) and potential ecological risk index (PERI). In addition, sediment quality was assessed taking into account the SQGs (sediment quality guidelines) (Abdullah et al., 2015; MacDonald et al., 2000; Mohajane & Manjoro, 2022; Ng et al., 2021). Human health risks associated with exposure to contaminated sediments were assessed using the RAGS (risk assessment guidance for superfund) methodology (USEPA, 1989, 2004).

Contamination factor (CF)

This factor evaluates the state of contamination of the sediment by each metal(loids) investigated. It measures the ratio between the concentration of each metal in the sediment and compares it with the background value, which is considered as the average concentration of the metal in the continental crust (Hakanson, 1980). The CF was calculated using Equation 1.

$$CF = \frac{C_{(metal)}}{C_{(background)}} \qquad (1)$$

Where, CF is the contamination factor, C_m is the metal concentration in the sediment sample, and C_b is the background concentration of metal in the upper continental crust. The background value for Hg was set at 0.085 mg/kg (Lide, 2008) and 1.8 mg/kg for As (Rumble, 1989). The following categories were used for the rating of the CF. A CF < 1 (low pollution), 1 ≤ CF < 3 (moderate pollution), 3 ≤ CF < 6 (considerable pollution) and CF > 6 (very high pollution) (Hakanson, 1980; Ng et al., 2021).

Geoaccumulation index (Igeo)

This Igeo index is a method used to assess the level of metal(loids) contamination in sediment. It establishes the difference in recorded metal concentrations between collected samples and naturally occurring background values in the upper continental crust (Li et al., 2016; Mohajane & Manjoro, 2022). It is calculated using Equation 2.

$$I_{\mathrm{geo}} = \log_2 \frac{C_n}{1.5 \ \mathrm{x} \ B_n} \qquad (2)$$

Where, C_n is the metal concentration in the sediments, B_n is the metal concentration in the upper continental crust, and 1.5 is a factor applied to minimize the effect of possible variations in background values. Igeo is classified into categories according to level, not polluted (level 0; Igeo ≤ 0), not polluted to moderately polluted (level 1; 0 < Igeo < 1), moderately polluted (level 2; 1 < Igeo < 2), moderately to heavily polluted (level 3; 2 < Igeo < 3), heavily polluted (level 4; 3 < Igeo < 4), heavily to extremely polluted (level 5; 4 < Igeo < 5) and extremely polluted (level 6; Igeo > 5) (Ferreira et al., 2022; Şimşek et al., 2022).

Pollution load index (PLI)

The pollution load index (PLI) is used to examine the degree or total level of metal(loids) contamination in sediments. It evaluates the level of contamination of each sample by considering the total number of metal(loids) investigated. Therefore, the PLI value provides an overall indication of the toxicity of a metal(loids) in the sediment (Abdullah et al., 2015). PLI was calculated using Equation 3.

$$\mathrm{PLI} = (\mathrm{CF1} \times \mathrm{CF2} \times \mathrm{CF3} \times \ldots \mathrm{CFn})^{1/\mathrm{n}} \qquad (3)$$

Where, CF corresponds to the contamination factor for each metal evaluated calculated as indicated in equation 1, and n is the number of metals evaluated. For its qualification and/or interpretation, the following classes and/or categories were used: 1 > PLI (not contaminated), PLI=1 (indicates presence of basic levels of contaminants) and 1 < PLI (progressive deterioration of the ecosystem and site quality) (Tomlinson et al., 1980).

Potential ecological risk index (PERI)

We first assessed the toxic risk taking into account the international sediment quality guidelines (SQGs). Sediment Hg and As concentrations found at each station were compared with threshold and/or limit effect concentration (TEC) values and probable effect concentration (PEC) values. Values at or below TEC suggest low probability of occurrence of toxic effects on aquatic biota, while values at or above PEC suggest high probability of occurrence of toxic effects on biota. The TEC for

Hg was set at 0.18 mg/kg and the PEC at 1.06 mg/kg, and for As TEC (9.70 mg/kg) and PEC (33.0 mg/kg) (MacDonald et al., 2000).

We then evaluated the ecological risk using the potential ecological risk index (PERI). This index allows us to evaluate the ecological risk generated by the contamination of sediments with toxic metals(loids) according to the toxicity of each metal investigated, and to the reaction of the environment. We calculated the coefficient of potential ecological risk (CPERi), which relates the toxicity level of each metal and its possible behavior in the environment. For this we used the Hakanson toxicity coefficient (HTC) or also known as the toxic response factor defined as 40 for Hg and 10 for As (Hakanson, 1980). The CPERi and PERI for each metal were calculated using formulas 4 and 5, respectively. The PERI represents the sum total of the potential risk for each metal investigated (Abdullah et al., 2015). The criteria used for the evaluation of this index are presented in Table S3.

The CPERi is the result of the multiplication of HTC by fi which is the same CF (contamination factor) determined by formula 1. On the other hand, the PERI is the sum of the potential risk of all the evaluated metal(loids) (Kusin et al., 2018). The CF, TEL, PEC and PLI were used to evaluate the severity of contamination in the sediments.

$$CPER_i = HTC\ x\ CF \qquad (4)$$

$$PERI = \sum CPER_i \qquad (5)$$

Human health risk assessment

In this study, health risk was estimated through three of three routes of exposure, oral ingestion also known as non-food ingestion, inhalation and dermal contact. Hg was used to estimate non-carcinogenic risks and As for carcinogenic risks for adults and children. Overall, risk was calculated by chronic daily exposure-CDE/mg/kg/day for all three routes of exposure (Canpolat et al., 2022; Ng et al., 2021).

The RAGS (risk assessment guidance for superfund) methodology suggested by USEPA was used to calculate the CDE. Formulas 6, 7, and 8 were used to calculate the CDE for the different routes of exposure, oral ingestion (CDE_{ing}), inhalation (CDE_{inh}) and dermal contact (CDE_{derm}) (USEPA, 1989, 2004).

$$\mathrm{CDE_{ing}} = \frac{C_{\mathrm{sed\ x\ IngR\ x\ EF\ x\ ED\ x\ CF}}}{\mathrm{BW\ x\ AT}} \quad (6)$$

$$\mathrm{CDE_{inh}} = \frac{C_{\mathrm{sed\ x\ InhR\ x\ EF\ x\ ED}}}{\mathrm{PEF\ x\ BW\ x\ AT}} \quad (7)$$

$$\mathrm{CDE_{derm}} = \frac{C_{\mathrm{sed\ x\ SA\ x\ AF\ x\ ABS\ x\ EF\ x\ ED\ x\ CF}}}{\mathrm{BW\ x\ AT}} \quad (8)$$

Where, C_{sed} is the concentration of metal(loids) in the sediment sample (mg/kg), CDE_{ing} is the ingestion exposure (mg/kg/day), CDE_{inh} is the inhalation exposure, and CDE_{derm} is the dermal exposure (mg/kg/day) (Şimşek et al., 2022; USEPA, 1989). The criteria used for the prediction of CDEs are presented in Table S4.

The hazard quotient (HQ) and hazard index (HI) were used for the evaluation of non-carcinogenic risks (USEPA, 2004). HQ indicates the non-carcinogenic risks, while HI expresses the cumulative non-carcinogenic risks, and is obtained by summing the hazard quotients, i.e. HQs (Dong et al., 2023; Wang et al., 2015). The HQ and HI were calculated using equations 9 and 10, respectively.

$$\mathrm{HQ} = \frac{\mathrm{CDE}}{\mathrm{RfD}} \quad (9)$$

$$\mathrm{HI} = \sum \mathrm{HQ} = \mathrm{HQ_{ing}} + \mathrm{HQ_{inh}} + \mathrm{HQ_{derm}} \quad (10)$$

In equation 9 the RfD (3.0 Hg mg/kg/day) represents the reference dose set to predict the risk to human health from exposure to contaminated sediments (USEPA, 2023b). If the HQ and HI values are greater than 1, the risk of occurrence of non-cancer adverse effects is high, whereas if these values are less than 1, the risk is considered low or negligible (USEPA, 2002). In general, the higher the HQ and HI values, the greater the health risk (Dong et al., 2023).

Here the total lifetime cancer risk (TLCR) was estimated only for As, because this metalloid has a carcinogenic slope factor. The TLCR refers to the probability that a person will develop some type of cancer during his or her lifetime related to total exposure to carcinogenic substances. This was estimated for the three routes of exposure, ingestion (LCR_{ing}), inhalation (LCR_{inh}) and dermal contact (LCR_{derm}) (USEPA, 1989). Equations 11 and 12 were used to calculate the TLCR.

$$\mathrm{LCR} = \mathrm{CDE} \times \mathrm{CSF} \qquad (11)$$

$$\sum \mathrm{TLCR} = \mathrm{CLR_{ing}} + \mathrm{CLR_{inh}} + \mathrm{CLR_{derm}} \qquad (12)$$

Where, CSF is the cancer slope factor, which has been defined for As at 1.5 mg/kg/day (USEPA, 1989). For the TLCR the USEPA has suggested a threshold of 1×10^{-4}, and an acceptable range of 1×10^{-6} to 1×10^{-4}, i.e. values within this range are considered negligible or acceptable risk, and higher risk values suggest high risk (USEPA, 2002).

Data analysis

The collected information was analyzed using the above-mentioned indices. To determine differences in Hg and As concentrations among the sampling sites, a one-way analysis of variance (ANOVA) was conducted after verifying the normality of the concentration data. Descriptive statistics, including mean, standard deviation, maximum, and minimum values, were reported. Multiple comparisons were performed using the Tukey post hoc test, with a significance level of $p < 0.05$. The sampling sites were further grouped based on the behavior of Hg and As concentrations using cluster analysis. Ward's method with Euclidean distance was employed to construct a dendrogram. GraphPad Prism 8.0 and Statgraphic Centurion XVI were the statistical software used for ANOVA and cluster analysis, respectively (Custodio et al., 2022; Varol, 2019).

Results

Total mercury and arsenic concentrations in sediments

The lowest mean for Hg concentrations was recorded at station E (0.09 ± 0.04 mg/kg) and the highest at station A (0.23 ± 0.07 mg/kg). The lowest concentration for As was recorded at station A (0.59 ± 0.16 mg/kg) and the highest at station B (2.68 ± 0.49 mg/kg). At stations located in the upstream (A, B, and C), the concentrations of Hg exceeded twice the global crustal background levels of 0.085 mg/kg (Lide, 2008). Furthermore, at these same stations, Hg concentrations exceeded twice the background levels reported for Colombia, which is 0.077 mg/kg (Rúa et al., 2014), except for station A where concentrations were found to exceed three times this value.

Among the five stations studied, only stations B, D, and E exceeded the global background value for As (1.80 mg/kg) (Rumble, 1989). The mean concentrations of Hg and As in sediments collected from different stations in the Atrato River basin are presented in Table 1. Additionally, analysis of variance revealed statistically significant differences in Hg (p = 0.02) and As (p = 0.03) concentrations among the sampling stations (Table S1). Specifically, Hg concentrations at station A (Figure 3a) were significantly different from the other stations ($p < 0.05$), and As concentrations at station B (Figure 3b) also exhibited significant differences.

Table 1. Descriptive statistics (mean, standard deviation-SD, range = minimum and maximum) of Hg and As concentrations in sediments collected at different stations in the Atrato River basin.

	Hg (mg/kg)		**As (mg/kg)**	
Stations	**Mean ±SD**	**Range**	**Mean ±SD**	**Range**
A	0.23±0.07	0.14-0.28	0.59±0.16	0.45-0.77
B	0.16±0.04	0.12-0.20	2.68±0.49	2.11-3.02
C	0.20±0.02	0.17-0.23	0.76±0.10	0.67-0.88
D	0.12±0.03	0.09-0.15	2.19±1.10	1.29-3.43
E	0.09±0.04	0.05-0.13	2.12±0.39	1.78-2.55

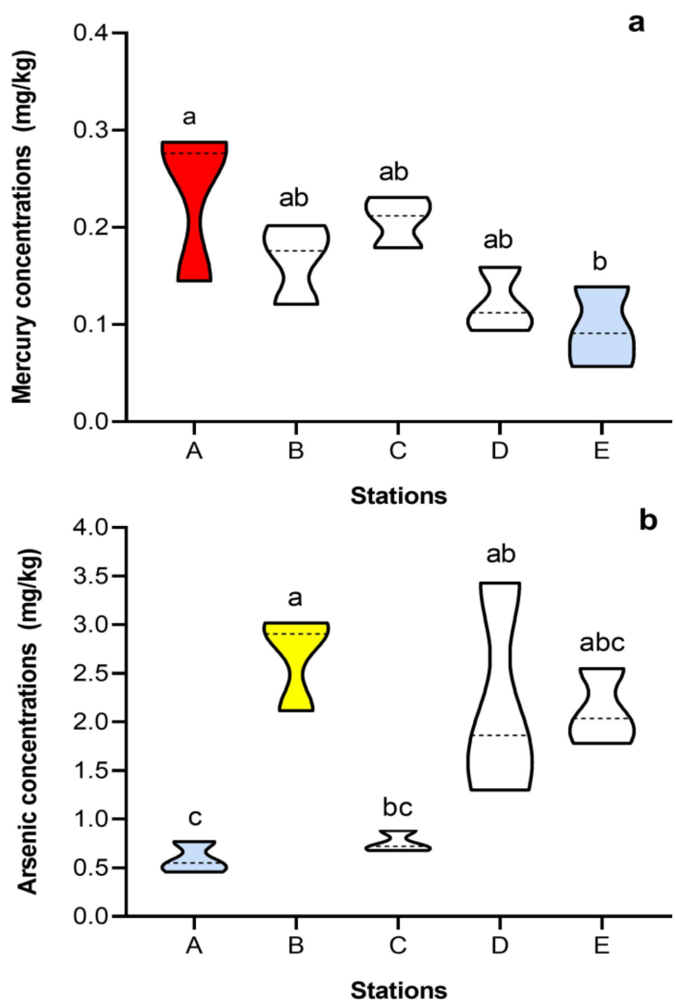

Figure 3. Comparison of mean Hg and As concentrations in sediments collected at different stations in the Atrato River basin. Means that do not share a letter indicate statistically significant differences ($p < 0.05$). Data are presented as violin plots, a combination of box and kernel density plots (Hintze & Nelson, 1998)

The cluster analysis further classified the Hg concentrations into three distinct groups. Stations B and C were grouped together (cluster 1) due to their similar concentrations, while stations D and E formed another group (cluster 2) with the lowest concentrations. Station A formed a separate group (cluster 3) because it exhibited the highest average concentrations of Hg among all the stations (Figure S1, Table 1). Regarding As concentrations, stations A and C were grouped together (cluster 1) as they had the lowest concentrations among all the stations. Stations D and E formed another group (cluster 2) with similar As concentrations. Station B, on the other hand, formed an independent group (cluster 3) with the highest concentration of As (Figure S1, Table 1).

Risk assessment

Severity of sediment contamination

The contamination factor (CF) for Hg concentrations ranged from 1.0 to 3.0 through all sediment samples, indicating that all samples had a moderate ($1 \leq CF < 3$) to considerable ($3 \leq CF < 6$) level of contamination with this metal. As expected, station A exhibited a considerable level of contamination ($CF = 3$) since Hg concentrations at this site were higher compared to the other stations (Tables 1 and 2). Regarding As concentrations, the CF values ranged from 0 to 1, indicating a contamination level between low ($CF < 1$) and moderate ($1 \leq CF < 3$). Stations A and C showed a low level of contamination with a CF=0, while stations B, D, and E had a moderate level of contamination (CF=1).

Based on the PLI values, most stations did not indicate contamination with Hg and As, except for station B (Figure 4). According to this index, the ecosystem at station B exhibited a significant deterioration of ecological quality. When sediment quality was evaluated using the SQGs, Hg and As concentrations at all stations were below the PEC values (Table 1). This indicates that during the sampling period, these elements did not pose a risk of harmful effects, particularly to benthic biota. However, at stations A and C, Hg concentrations were slight above the TEC values, suggesting the possibility of a toxic risk in these areas. On the other hand, As concentrations at all stations were below both TEC and PEC values (Figure S2, Table 1), indicating that this metalloid did not represent a toxicological risk during the sampling period.

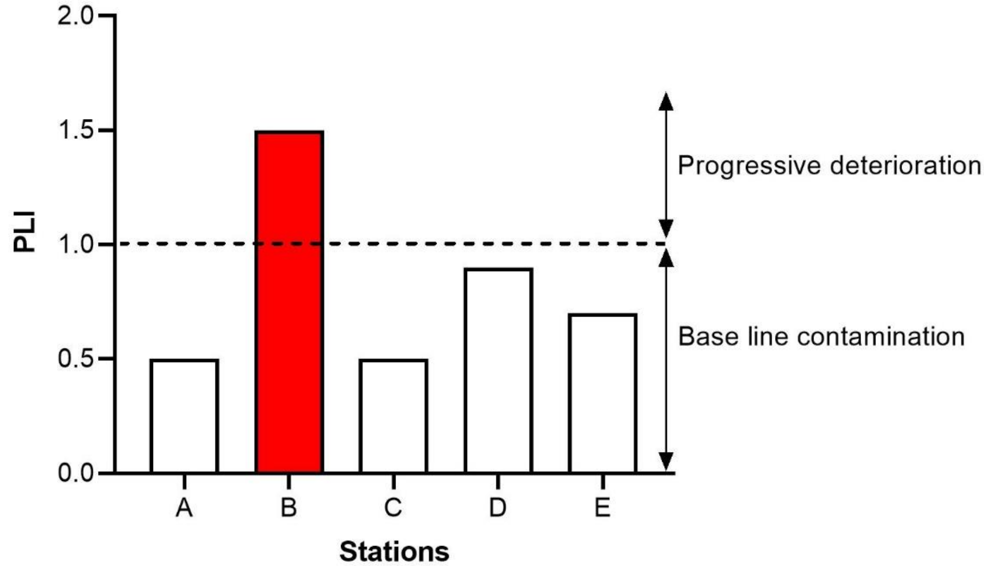

Figure 4. Evaluation of sediment contamination collected at different stations in the Atrato River with respect to the pollution load index-PLI.

Geoaccumulation and potential ecological risk index

The Igeo values for Hg at stations at the upper zone (A, B, and C) were classified as level 2 (1 < Igeo < 2), indicating moderate contamination of sediments with Hg. Meanwhile, stations D and E were categorized in class 1 (0 < Igeo < 1), suggesting slightly contamination with this metal. As Igeo for As, all sediment samples showed signs of contamination with this metalloid (0 < Igeo < 1) and exhibited an increasing trend. The evaluation results of Hg and As indices in sediments can be found in Table S2.

Table 2 shows the CPER and PERI calculation values for each of station. With regards to CPER for Hg, the values at all stations indicated contamination, with values exceeding 40. At stations close to the mouth of the river D and E the sediment were moderately contaminated, and the remaining stations A, B, and C were heavily contaminated with this metal. In contrast, the CPER values for As at all stations were <40, indicating that the sediments were low with this metalloid (Table S3). When we integrated the metals by PERI, this index revealed that at upstream stations (A, B and C) the ecological risk was high (80 ≤ PERI ≤ 160), whereas at downstream stations (D and E) was moderated (40 ≤ PERI ≤ 80).

Table 2. Potential ecological risk index calculated for each of the sampling stations in the Atrato River basin.

	CF		***CPER$_i$***			
Stations	**Hg**	**As**	**Hg**	**As**	***PERI***	**Level of risk**
A	2.8	0.3	112	3	115	high
B	2.0	1.5	80	15	95	high
C	2.4	0.4	96	4	100	high
D	1.1	1.2	44	12	56	moderate
E	1.4	1.2	56	12	68	moderate

Human health risk

Table 3 presents the values of the estimate of non-cancinogenic risk resulting from Hg contamination in sediments in the Atrato River basin. In general, HI>1, suggested risk of significant non-carcinogenic adverse effects (Table 3). Depending on the sampling area, the HI values indicated a higher risk for children compared to adults. Specifically, the estimated HI for children residing in the upper zone (i.e. agricultural area, close to the mouth) was 8.8 E-07, which was higher than the HI (4.7 E-07) for children residing in the lower zone (i.e. gold mining area). Similarly, the HI was higher (3.8E-07) for adults residing in the upper watershed compared to adults residing in the lower watershed (2.0E-07). This pattern indicates a greater non-carcinogenic risk for individuals living in the upper zone.

Table 3. Chronic daily exposure values (CDE), hazard quotient (HQ) and hazard index (HI) for non-carcinogenic risks resulting from Hg concentrations in sediments collected at different stations in the Atrato River basin (ing: ingestion; inh: inhalation; derm: dermal). For the CDE calculations, values of exposure factors from Table S4 were used.

Basin	CDE_{ing}	CDE_{inh}	CDE_{derm}	HQ_{ing}	HQ_{inh}	HQ_{derm}	HI
Upper zone							
Adult	1.1 E-06	2.2 E-10	4.4 E-08	3.7 E-07	7.4 E-11	1.5 E-08	3.9 E-07
Children	2.6 E-06	2.6 E-10	5.2 E-08	8.7 E-07	8.7 E-11	1.7 E-08	8.8 E-07
Lower zone							
Adult	6.0 E-07	1.2 E-10	2.4 E-08	2.0 E-07	4.0 E-11	8.0 E-09	2.1 E-07
Children	1.4 E-06	1.4 E-10	2.8 E-08	4.7 E-07	4.7 E-11	9.3 E-09	4.7 E-07

Routes of exposure were distributed in the following order of magnitude: in children (upper part of the basin) ingestion > inhalation > dermal contact >; children (lower part of the basin) dermal contact > ingestion > inhalation >. Adults (upper part of the basin) inhalation > ingestion > dermal contact >; adults (lower part of the basin) dermal contact > inhalation > ingestion >. With respect to carcinogenic risks, all TLCR values were in the acceptable range of 1×10^{-6} to 1×10^{-4} recommended by USEPA, indicating negligible cancer risk. Table 4 shows that the maximum TLCR values were higher in children (2.6 E-05 and 4.2 E-05) compared to adults (1.2 E-05 and 1.9 E-05), depending on the sampling area. According to this study, the main routes of exposure that could have the greatest impact on cancer risk in the riparian population are oral ingestion and dermal contact. The study suggests that these routes of exposure, particularly for children, contribute significantly to the potential cancer risk.

Table 4. Chronic daily exposure values (CDE) and calculation of total lifetime cancer risk (TLCR) resulting from As concentrations in sediments collected at different stations in the Atrato River basin (ing: ingestion; inh: inhalation; derm: dermal). dermal. For the CDE calculations, values of exposure factors from Table S4 were used.

Basin part	CDE_{ing}	CDE_{inh}	CDE_{derm}	LCR_{ing}	LCR_{inh}	LCR_{derm}	TLCR
Upper zone							
Adult	7.4 E-06	1.5 E-09	3.0 E-07	1.1 E-05	2.2 E-09	4.4 E-07	1.2 E-05
Children	1.7 E-05	1.7 E-09	5.7 E-08	2.6 E-05	2.6 E-09	8.6 E-08	2.6 E-05
Lower zone							
Adult	1.2 E-05	2.4 E-09	4.7 E-07	1.8 E-05	3.6 E-09	7.1 E-07	1.9 E-05
Children	2.8 E-05	2.8 E-09	5.5 E-07	4.1 E-05	4.1 E-09	8.3 E-07	4.2 E-05

Discussion

Mercury and arsenic concentrations in sediments

The maximum concentrations of Hg were recorded at stations A (0.23 mg/kg) and C (0.20 mg/kg). In contrast, stations B (2.68 mg/kg) and D (2.19 mg/kg) had the highest concentrations of As. When comparing the average concentrations, the overall average for As (4.32 mg/kg) was higher at downstream stations compared to the stations located upstream (4.03 mg/kg). However, Hg concentrations at all stations were relatively low and showed little variability (Table 1, Figure 5). Other studies in the area have also reported relatively low and consistent Hg concentrations in river sediments (Palacios-Torres et al., 2018) as well as an increase in As concentrations downstream (Palacios-Torres et al., 2020).

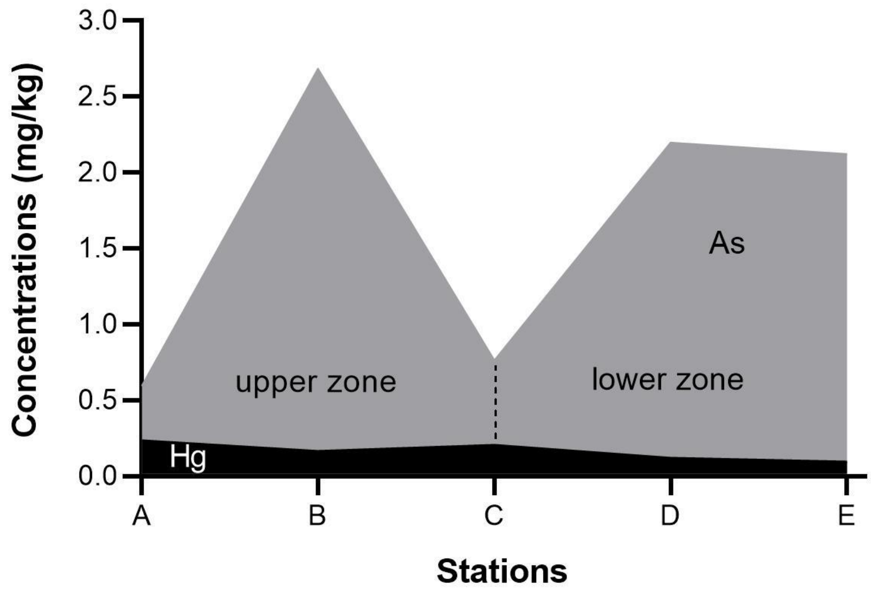

Figure 5. Distribution of mean Hg and As concentrations in sediments collected at different stations in the Atrato River basin.

In aquatic environments, particularly tropical ones, Hg concentrations in sediments tend to be lower as they move away from the source. This is partly explained in the fact that the high flow and high rainfall that occur in these ecosystems facilitate sediment entrainment and metal(loids) dilution reducing to some extent the anthropogenic impact (Barletta et al., 2019; Da Silva et al., 2019; Haris et al., 2017; Luo et al., 2020). Additionally, Hg in its metallic form tends to have low mobility and to concentrate at sites close to the source (Gutiérrez-Mosquera et al., 2020), which is partly influenced by organic matter, as this can help to reduce Hg particulate loading, mainly downstream (Eckley et al., 2021), but it can also affect the toxicity, distribution, solubility and bioavailability of metal(loids) in sediments (Pinedo-Hernández et al., 2015; Zonta et al., 2019).

The study area in the Atrato watershed is facing threats from both gold mining and agriculture, which pose risks to the natural ecosystems (Palomino-Ángel et al., 2019). However, it is worth noting that the intensity of artisanal and mechanized gold mining is higher in the upper part of the Atrato River basin, while agriculture is more prevalent in the lower zone. This distribution pattern helps explain the localized contamination observed along the river (Gallego et al., 2018; UNODC, 2016).

Research conducted worldwide supports the concept that As contamination in aquatic environments is often driven by anthropogenic sources, particularly agriculture. This is primarily due to the presence of As in various agrochemicals commonly used in agricultural practices. The application of these agrochemicals contributes to increased concentrations of As in aquatic ecosystems (Badrzadeh et al., 2022; Wang et al., 2023; Xiao et al., 2022).

Ecological and human health risk assessment

Ecological risk

The sediments were classified by the CF as moderately (stations B, D, and E) to significantly contaminated with Hg (station A), and as low (stations A and C) to moderately contaminated (stations B, D, and E) with As. Furthermore, the Igeo values revealed that the sediment samples showed moderate contamination with Hg and low contamination with As, with variations depending on the sampling zone (Table S2).

Moreover, the concentrations of Hg and As were slightly higher than the background values, with the exception of stations A and C for As. This suggests that contamination in the Atrato watershed is primarily influenced by anthropogenic processes. Previous studies (Palacios-Torres et al., 2018; Salazar-Camacho et al., 2021) support this observation, highlighting the significant impact of human activities on the contamination levels. Notably, sampling stations with similar Hg and As concentrations indicate a common source of contamination (Hasan et al., 2023; Varol, 2019). By integrating Hg and As concentrations using the PLI, it becomes evident that all sediments were contaminated above the background levels of metal(loids). However, at station B, the PLI revealed a progressive degradation of the ecological quality of the site, suggesting a more pronounced impact of contamination (Figure 4).

During the collection of sediment samples, clear evidence was observed of mining activities involving heavy machinery in the vicinity of station B. This observation provides a possible explanation for the degraded ecological quality found at this particular site. The findings support the notion that mining activities not only affect the local site but also have implications for the overall ecological health of the Atrato River as a complex system (Gutiérrez-Mosquera, Shruti, et al., 2018; Marrugo-Madrid et al., 2022; Marrugo-Negrete et al., 2023).

The lower PLI values at all stations, except for station B, can be attributed to the global approach employed by the PLI. The PLI is an index that evaluates the pollution status of each site, considering the total number of pollutants analyzed independently of the concentrations of each pollutant. In addition, this index takes into account the pollution factor of each pollutant i.e. it compares the concentrations found with the background concentrations, providing an overall idea of the pollution status at a given site (Mohajane & Manjoro, 2022; Tomlinson et al., 1980). In this context, the PLI serves as a practical and valuable tool for identifying and managing sites with significant degradation, providing a holistic perspective on pollution levels, enabling the identification of problematic areas that require remediation and/or targeted management strategies (Ferreira et al., 2022; Hasan et al., 2023).

In agreement with the SQGs, Hg and As concentrations were lower than the PEC values (1.06 Hg mg/kg, 33.0 As mg/kg) at all sampling stations, suggesting that risks of toxic effects to aquatic organisms were unlikely. This finding for As was in agreement with that reported by Palacios-Torres et al., (2020) who also found that sediment As concentrations in the Atrato River when contrasted with the SQGs were lower than TEC (9.7 As mg/kg) and PEC (33.0 As mg/kg) indicating that toxic effects on biota were unlikely. However, when these same authors examined the PERI results, some sampling sites represented a strong ecological risk, similar to what this index revealed in the present study (Table 2).

In our study, it was found that Hg concentrations in two of the five stations (A and C) exceeded the TEC. This finding contrasts with the previous report by Palacios-Torres et al., (2018) for the same study area, where Hg concentrations were consistently below the TEC (0.18 mg/kg) in all evaluated sites. The divergence in results suggests that Hg contamination in sediments within the Atrato River may be increasing over time (BadrElDin et al., 2022; Hasan et al., 2023). The progressive bioaccumulation of toxic metal(loids) in biota may result in harmful effects (Varol & Sünbül, 2018; Zonta et al., 2019).

Indeed, values above or below the TEC serve as indicators of sediment quality. When concentrations exceed the TEC, it suggests poor sediment quality and potential ecological risks. Conversely, concentrations below the TEC indicate better sediment quality and reduced ecological risks. Considering the TEC criterion on an individual basis can provide a reliable basis for assessing the ecological quality of freshwater systems (Bjørgesæter & Gray, 2008; MacDonald et al., 2000). It is also a simple and useful method for guiding environmental conservation programs (Bjørgesæter & Gray, 2008).

Risk to human health from exposure to contaminated sediments

In general, the HI values were found to be greater than one, indicating a potential risk of non-cancer associated with sediment contamination (USEPA, 2004, 2023b). The contributions of exposure routes varied depending on the sampling area, but oral ingestion and inhalation were identified as the primary routes impacting the final HI score (Table 3). These results are consistent with similar studies conducted in different parts of the world, such as (Alves et al., 2014), India (Adimalla et al., 2020), Argentina (Magni et al., 2021), Peru (Custodio et al., 2022), Turkey (Şimşek et al., 2022) and China (Jiang et al., 2023; Wang et al., 2015). Furthermore, these studies also reported a higher risk for children compared to adults, which is a trend observed in the present study as well (Table 3).

The estimated carcinogenic risk, indicated by the TLCR values, for both children and adults fell within the acceptable range (1×10^{-6} a 1×10^{-4}), suggesting that the cancer risk from exposure to As-contaminated sediments was negligible (USEPA, 2002, 2004). However, it is dermal contact and oral ingestion obtained the highest values in the final TLCR score, particularly in children (Table 4). These results are consistent with the study by Canpolat et al., (2022), which also found that oral ingestion and dermal contact were the primary exposure routes contributing to the TLCR for As-contaminated sediments in the northwestern region of Turkey.

Similar results have been reported in various regions worldwide, where oral ingestion and dermal contact were identified as the two main pathways determining the risk of cancer from exposure to sediments contaminated with toxic metal(loids). Furthermore, based on the TLCR values reported in these studies, children may be more susceptible to developing certain types of cancer over their lifetime (Custodio et al., 2022; Kusin et al., 2018; Magni et al., 2021).

The increased vulnerability of children to the effects of chemical contamination can be attributed to numerous habits they may develop. For instance, unintentional ingestion of water while swimming in rivers or the habit of sucking their fingers can contribute to higher exposure levels (Hoang et al., 2021; Özkaynak et al., 2022). As a result, children have a greater likelihood of ingesting contaminated soil and dust, thereby exposing themselves to higher carcinogenic and non-carcinogenic risks compared to adults (Luo et al., 2012; Varol, 2019).

Researchers have considered that risks from sediment exposure should not go unnoticed, as the absence of risk reported by mathematical equations does not necessarily reveal the combined effects that toxic substances could cause on the environment and human health (Ali & Khan, 2018a; Price, 2023; K. S. Vieira et al., 2021). Therefore, having assessed human health risk by exposure to an unusual matrix such as sediment, provides an interesting basis in this study.

Conclusions

In this study, we conducted measurements of Hg and As concentrations in sediments from the Atrato River and assessed the potential risks they pose to both the ecosystem and human health. Our findings revealed that Hg concentrations consistently exceeded both global (0.085 mg/kg) and local (0.077 mg/kg) background levels. In contrast, As concentrations exceeded background levels (1.8 mg/kg) at only three out of the five stations (B, D, and E). Moreover, the levels of Hg and As concentrations varied depending on the sampling zone, indicating the influence of different anthropogenic activities on the ecological quality of the ecosystem.

The assessment of sediment contamination using indices such as Igeo, PLI, and PERI indicated moderate to significant contamination levels. For instance, PERI values suggested a high ecological risk due to Hg and As contamination at stations A, B, and C, while the risk was moderate at stations D and E. Furthermore, HI values greater than 1 for non-carcinogenic risks indicated a significant risk of exposure to Hg-contaminated sediments for the riparian population. Oral ingestion and inhalation were identified as the main routes of concern, particularly for children. On the other hand, the estimated TLCR values fell within the tolerable or acceptable risk range (1×10^{-6} to 1×10^{-4}), indicating no significant carcinogenic risk. However, dermal contact (8.5E-08) and oral ingestion (4.1E-05) were the routes that yielded the highest values in the TLCR estimation, primarily observed in children and varied depending on the sampling area.

These results can serve as a reference for risk assessments related to exposure to contaminated environmental matrices, which may not initially pose a risk to human health. However, it is important to note that future work in the study area should include increasing the number of samples and the analysis of bioavailable Hg and As species that would allow strengthening the interpretation of results. Such efforts would help improve the accuracy and understanding of the potential risks associated with sediment contamination in the Atrato River.

Chapter 3

Supplementary Data

Table S1. Results of the analysis of variance for Hg and As concentrations (mg/kg) in sediments collected at different stations in the Atrato River basin.

Source of variation	Sum of squares	Degrees of freedom	Mean sum squares	P value
Concentrations (Hg)	0.041	4	0.010	0.025
Residual	0.023	10	0.002	
Total	0.064	14		
Concentrations (As)	10.46	4	2.615	0.003
Residual	3.311	10	0.3311	
Total	13.77	14		

Table S2. Geoaccumulation index (Igeo) of Hg and As in sediments collected at different stations in the Atrato River basin.

	Igeo	
Stations	**Hg**	**As**
A	1.85	0.22
B	1.31	0.99
C	1.63	0.28
D	0.95	0.81
E	0.75	0.79

Table S3. Classification of potential ecological risk (PERI) (Hakanson, 1980).

PERI	Level of risk
< 40	low
40-80	moderate
80-160	high
160-320	very high
> 320	extremely high

Table S4. Exposure factors used in CDE prediction (USEPA, 1989, 2004).

Parameter	Definition (unit)	Amount
IngR	Ingestion rate (mg/day)	100 (adult), 200 (children)
InhR	Inhalation rate (mg/cm^2)	20
EF	Exposure frequency (days/year)	350
ED	Exposure duration (years)	24 (adult), 6 (children)
CF	Conversion factor (kg/mg)	1×10^{-6}
PEF	Particle emission factor (m^3/kg)	1.36×10^9
BW	Body weight (kg)	70 kg (adult), 15 kg (children)
AT	Averaging time (days)	2190
SA	Surface area (cm^2)	5700
AF	Adherence factor (mg/cm^2)	0.07
ABS	Dermal absorption factor (unitless)	0.01

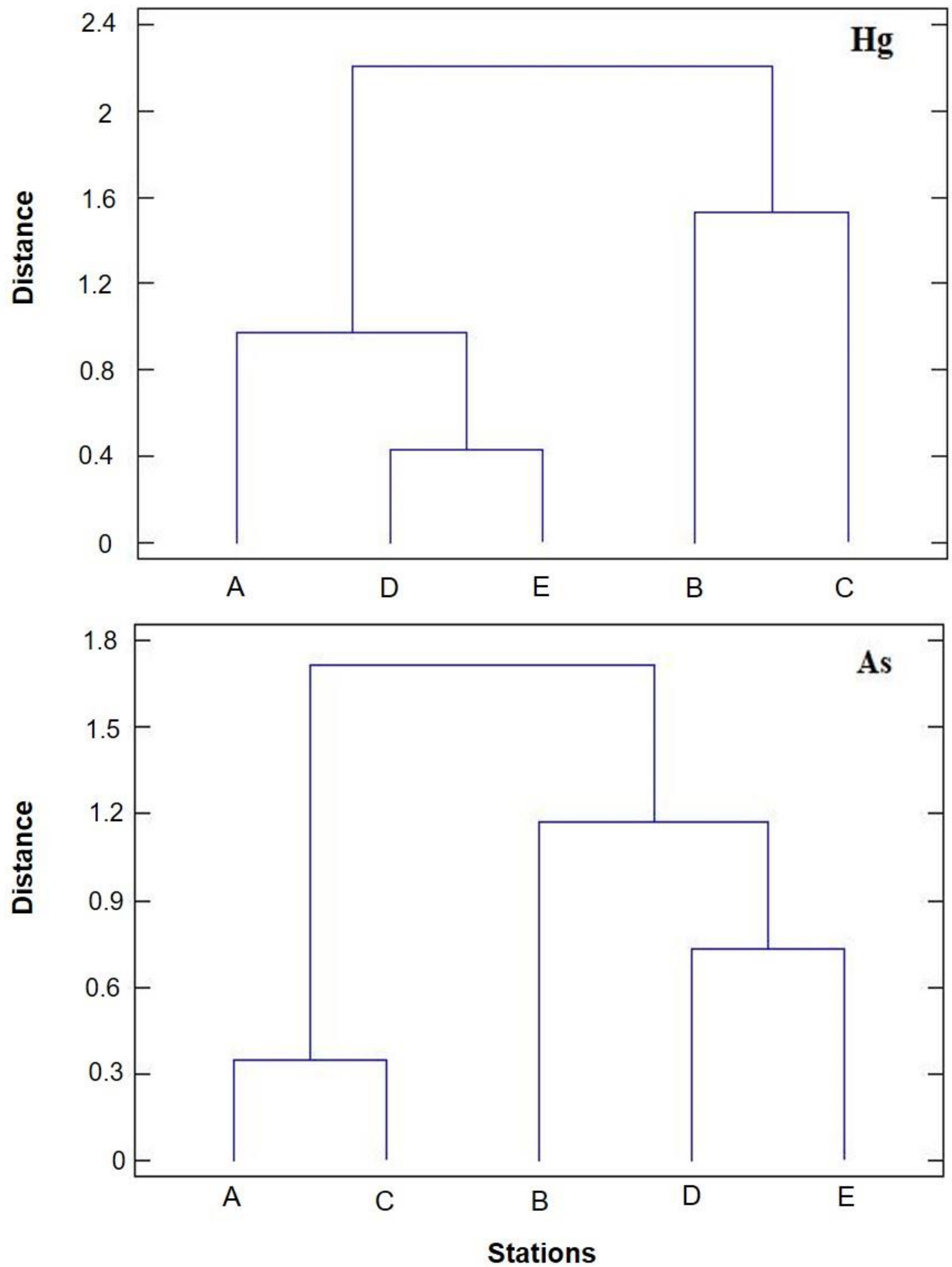

Figure S1. Dendrogram of the clustering of sampling sites according to Hg and As concentrations recorded in sediments collected at different stations in the Atrato River basin.

Hg					
Stations	A	B	C	D	E
As					

Figure S2. Classification of sediment quality collected at different stations in the Atrato River basin with respect to TEC and PEC values. Cells in gray indicate concentrations below TEC and PEC, and cells in red indicate concentrations above TEC.

Overall conclusions and outlook

General conclusions

Anthropogenic chemical pollution is a driving force behind complex changes that threaten human and ecosystem health. Therefore, measuring and monitoring concentrations of hazardous chemicals constitutes an important basis for local environmental management, as it helps describe and understand the associated impacts. These tasks are also essential, because they provide tools for the development of predictive models that allow for timely reporting of potential risks derived from contamination. In a constantly changing world, characterizing and understanding the complexity of pollution and biological responses can be useful in developing new policies to protect human health and biodiversity. However, achieving the objectives requires an interdisciplinary analytical framework to understand the results from a comprehensive and systemic perspective, which could help identify the best solution paths.

Limitations

This study provides concrete evidence of anthropogenic chemical contamination in the Atrato River basin. In addition to highlighting the contamination occurring in this watershed, we also report on the toxic effects imposed by sediment and water column contamination. However, due to budgetary constraints we were unable to identify arsenic species and the factors that influence the bioaccumulation of this metalloid including dissolved organic matter and phosphorus concentrations.

We also failed to measure contents of organic contaminants including pesticides and other toxic metals (e.g. Pb, Cd, and Cu), which could be involved in genotoxic effects in biota. Taking these aspects into account and studying genotoxic effects on biota on a larger scale, i.e. increasing the sample size of fish and other organisms, may help to clarify the results of this study more precisely.

Future outlook

Studies on contamination, especially with toxic metals(loids), at local, regional and global levels have mostly focused on the measurement of total concentrations in environmental and biological matrices. However, it is rare that chemical speciation and bioavailability of the various more toxic forms of metals are assessed. This calls for increased efforts to establish more precisely the associated eco-toxicological risks.

In this regard, we encourage future researchers to focus their attention on: (1) assessing speciation mechanisms and environmental factors (e.g. dissolved organic matter and phosphorus) that influence arsenic assimilation, as well as the bioavailability of other toxic metals such as cadmium, lead, copper, and persistent organic pollutants including pesticides, (2) investigate in depth the genotoxic effects on biota and their relationship with the concentrations of contaminants present, (3) Finally, we suggest developing health and biodiversity management policies with a differential approach, i.e. that collect scientific evidence that explains the behavior of pollution in a specific site. This will partly guarantee the effectiveness and positive impact of policy instruments for the control and management of hazardous metal(loids) contamination.

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