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Bioaccumulation of mercury in direct-developing frogs: The aftermath of illegal gold mining in a National Park

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The use of mercury in mining gold is an illegal but still common practice in developing countries and is the world's largest source of mercury pollution. The mercury released into the environment bioaccumulates in organism tissues due to its chemical properties and can adversely alter wildlife's neurological and reproductive systems. Frogs are susceptible to mercury contamination from gold mining because of their high skin permeability and association with aquatic environments. However, the effect of mercury pollution on direct-developing frogs is poorly known, particularly in tropical highlands. To understand the impact of mercury due to gold mining contamination on biodiversity of Tropical Andes, we assessed the bioaccumulation of mercury on direct-developing frogs of genus *Pristimantis* in a montane forest. We assessed bioaccumulation by comparing muscle tissue samples of frogs and sediments of streams in an area previously affected by illegal gold mining inside the Farallones de Cali National Park. Even though gold mining has not been conducted in the area for several years, we found mercury in muscle samples of direct-developing species of genus *Pristimantis* and alarming mercury concentrations in the sediment samples that exceed risk thresholds according international guidelines of the WHO (1.0749 $\mu g.g^{-1}$) and countries such as Canada, USA and Brazil (0.35 $\mu g.g^{-1}$). Our results suggest that the use of heavy metals in the gold mining can affect non-aquatic species causing bioaccumulation of heavy metals, which can be an important threat to wildlife populations, the stability of the ecosystem, and public health.

Keywords: Andean forests, mercury pollution, muscle tissue, streams pollution, sediments, total mercury

INTRODUCTION

Environmental pollution of heavy metals has become of great concern due to the adverse effects on biodiversity and public health worldwide. The mercury-based gold mining industry is the world's largest source of mercury pollution, with emissions from 410 to 1,400 tons of mercury each year, accounting for 37 % of global mercury emissions (Esdaile & Chalker, 2018). Despite technological advancements that reduce environmental impact and make mining practices more sustainable (Pantoja-Timarán et al., 2005; Rojas-Cruz & Mejía-Tobón, 2007), the use of mercury to amalgamate and concentrate precious metals is still a common practice in the mining industry of developing countries due to low costs and ease of use (Pantoja-Timarán et al., 2005; Rojas-Cruz & Mejía-Tobón, 2007; Hernandez-Cordoba et al., 2013). During artisanal and small-scale gold mining, mercury is released into rivers and streams and deposited in sediments, and methylmercury is produced, which is the most toxic and common organic mercury compound found in the environment (Pinedo-Hernandez et al., 2015; Betancur-Corredor et al., 2018). Mercury deposition in natural environments impacts the stability of ecosystems because of the reproductive and neurological consequences of exposing organisms (Fitzgerald et al., 1991; Bank et al., 2007).

Mercury bioaccumulates in organisms' tissues due to its recalcitrant properties and high affinity for organic matter and sulphur compounds (Pinedo-Hernández et al., 2015). Furthermore, methylation of inorganic mercury provides an efficient transmission pathway in food webs; thus, it is biomagnified and results in an increased risk for animals of high trophic levels (Bank, 2020). Thus, predators can be more susceptible to the adverse effects of mercury pollution. The accumulation of mercury in organisms' tissues causes behavioural, neurochemical, hormonal, and reproductive changes, directly affecting wildlife species' populations (Scheuhammer et al., 2007; Bergeron et al., 2011). Besides, methylmercury can easily pass through the blood-brain barrier and has a high affinity for brain tissue, decreasing antioxidant function and damaging the nervous system (Li et al., 2018).

The impact of bioaccumulation of mercury due to gold mining has been quantified in different organisms, including invertebrates and vertebrates (Ahumada, 1994; Bank et al., 2007; Alvarez et al., 2012; Zapata et al.,

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2014). Frogs, in particular, have great sensitivity to the concentration of pollutants due to their natural history traits, especially during the tadpole stage before limb formation (Rowe et al., 1996; Burger & Snodgrass, 2000). The absorption of contaminants such as mercury by frogs occurs through their permeable skin by direct contact with polluted water or sediments and by ingesting mercury-contaminated food (López-Noguera, 2015). Additionally, their trophic interactions play a crucial role in the biomagnification processes (Alvarez et al., 2012; Hernandez-Cordoba et al., 2013). Thus, frogs are excellent biological indicators for evaluating the effect of mercury in gold mining. However, the effect of mercury pollution on direct-developing frogs, which do not pass through the aquatic larval stages, is unknown.

In Colombian lowlands, mercury bioaccumulation has been reported for tadpoles in areas impacted by gold mining (Hernandez-Cordoba et al., 2013), as well as in other organisms such as fish (Alvarez et al., 2012) and turtles (Zapata et al., 2014). However, knowledge of gold mining's impacts on the highlands of the Colombian Andes, which are highly threatened by this practice, is lacking (Urbina-Cardona, 2011). Also, the mountain ecosystem of the Andes is the primary source of water supply for the most important rivers and hold a significant portion of the country's biodiversity with a high degree of endemism (Romero-Ruiz et al., 2008); thus, mercury pollution of these ecosystems can have a substantial impact on biodiversity and public health.

To understand the impact of mercury pollution due to gold mining contamination on biodiversity, we assessed the bioaccumulation of mercury on direct-developing frogs in a highly diverse forest. Hence, we took tissue samples of *Pristimantis* genus frogs and stream sediments in an Andean Forest previously impacted by illegal gold mining inside a National Natural Park (Farallones de Cali). We expected to find low mercury concentrations in tissues of the direct-developing frogs because of their independence from water sources where mercury is deposited. In addition, although large-scale mining has not been conducted in the area for several years, we expected to find high concentrations of mercury in the sediments of streams because of the excessive amounts of mercury used for gold mining throughout the area in the past.

MATERIALS & METHODS

Study area

This study was conducted in Alto del Buey - Minas del Socorro area at Farallones de Cali National Park, a protected area on the Colombian Western Andes in Valle del Cauca, Colombia (Fig. 1). Study locality has 196,364.9 ha that includes Tropical Rainforest, Sub-Andean Forest, High-Andean Forest, and Paramo ecosystems, and the site of born of the Cali River, one of the main water supplies for the municipality of Cali. Since the last century, Alto del Buey has been an area of

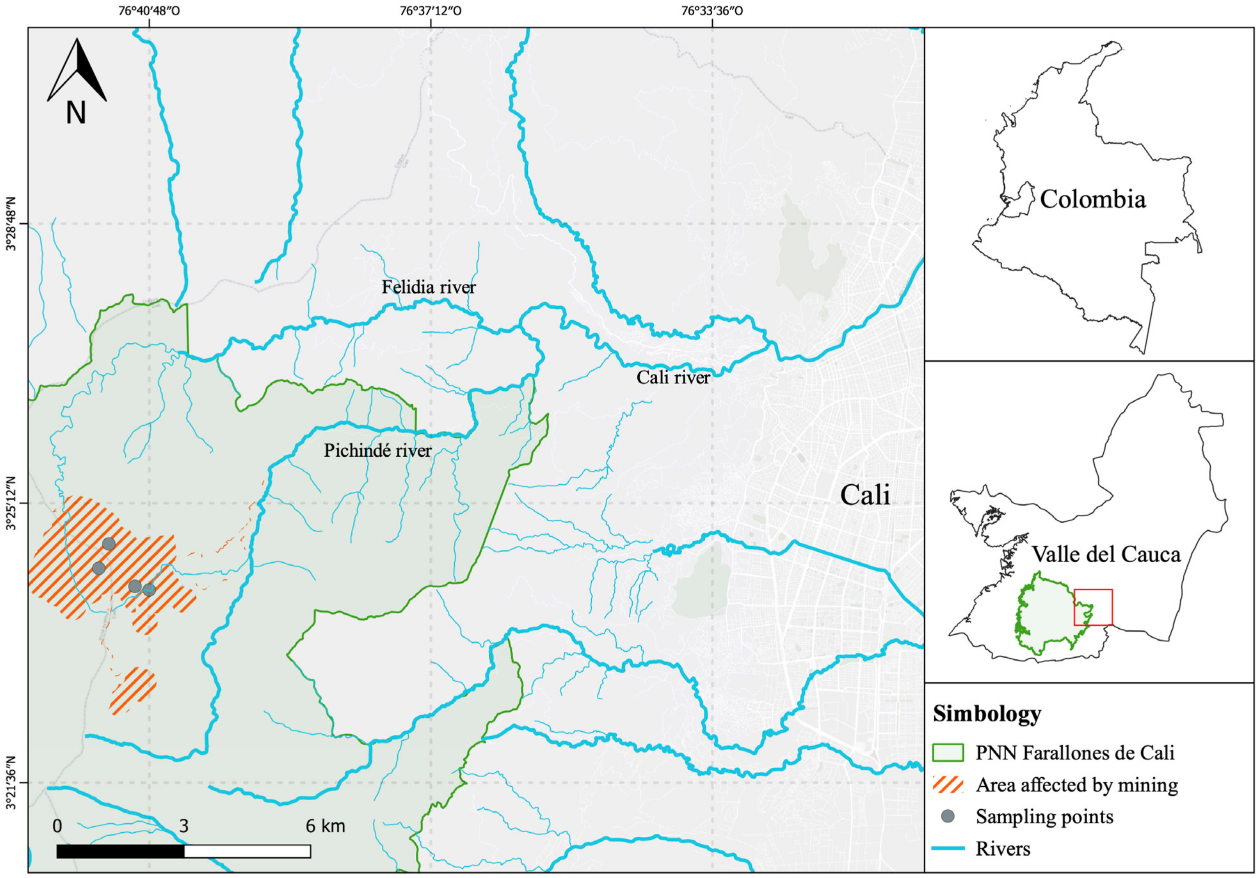

Figure 1. Alto del Buey study area (PNN Farallones de Cali, Valle del Cauca, Colombia), showing the location of the sampling points and the associated rivers.

illegal gold mining in the headwaters of the Felidia and Pichindé rivers, the main tributaries of the Cali River. Besides, no biological information from this area existed until a few years ago (e.g. Cuellar-Valencia et al., 2020; 2021).

Since 2010, park staff visits have evidenced the environmental deterioration caused by mining, which included 676.7 ha affected by more than 400 mining sinkholes, several camps, access roads that lead to deforestation, heavy machinery, and evidence of chemical contaminants used, such as cyanide and mercury that end up in water sources. Following the dismantling of the mining camps, the whole area is in natural regeneration.

Fieldwork

We sampled sites with noticeable effects of illegal gold mining inside the study area between September and December 2017, covering an altitudinal range between 3,000 and 3,500 m. The streams of four sites (El Feo, Campamento base, La Cruz, Sendero) were sampled in the study, exhibiting notable environmental deterioration in deforestation, chemical contamination, and solid waste despite two years without large-scale mining (Fig. 2); Thus, we looked for frogs along the streams using the visual encounter survey method (VES). We captured adult frogs and identified them to the lowest possible taxonomic level. Several individuals of the largest and most abundant species were transported alive to the facilities of the Universidad del Valle for further analysis. Additionally, we took a sediment sample from the streams where each individual was collected. Finally, we took an additional sediment sample from a site where a mill for gold mining had been located in the past. All sediment samples were handled with latex gloves and stored in hermetically sealed bags without being sieved and with some stream water.

Laboratory analysis

We assessed mercury accumulation for eight samples of four species of *Pristimantis*: *P. brevifrons* (1), *P. buckleyi* (3), *P.* aff *calcaratus* (2), and *P.* sp (2). To obtain these samples were needed 13 individuals, one for each sample for *P. buckleyi* (Campamento base) and *P.* aff *calcaratus* (Sendero), two individuals for the sample of *P. brevifrons* (El Feo) and three individuals for each sample of *P.* sp (La Cruz). All individuals that were used for one sample were collected in the same place. As the sediment samples were taken by each individual, the tissues samples where more than one individual was used, the sediment samples were combined to form a unique sample. To avoid overestimating the mercury measurement, we kept individuals alive 48 hours before analyses to eliminate their intestinal contents (Hernandez-Cordoba et al.,

Figure 2. Environmental impact caused by illegal gold mining at Alto del Buey, PNN Farallones de Cali: **a)** deforestation and mining camp remains; **b)** remains of heavy machinery used for mining; **c)** abandoned mining sinkhole; **d)** stream affected by chemical contamination (Arrows point to drops of liquid mercury in water).

Table 1. Total mercury concentration [Hg^{+2}] found in frog tissues and sediments, and bioaccumulation index (BI) of mercury in frogs collected at PNN Farallones de Cali, Colombia.

Species	Site	[Hg^{+2}] tissue ($\mu g.g^{-1}$)	[Hg^{+2}] sediment ($\mu g.g^{-1}$)	BI
Pristimantis sp	La cruz	0.0016	0.0053	-0.0037
Pristimantis sp	La cruz	0.0052	0.0105	-0.0053
Pristimantis brevifrons	El Feo	0.0027	0.0053	-0.0026
Pristimantis buckleyi	Campamento base	0.0177	0.4415	-0.4238
Pristimantis buckleyi	Campamento base	0.0046	0.2835	-0.2789
Pristimantis buckleyi	Campamento base	0.0426	2.200	-2.1574
Pristimantis aff *calcaratus*	Sendero	0.0004	0.0206	-0.0202
Pristimantis aff *calcaratus*	Sendero	0.0004	0.0588	-0.0584
-	El Feo	-	1.400[a]	-

[a] Sediment sample taken from a mill for gold mining

2013). Then each individual was euthanised following the protocol proposed by Cortez-F et al. (2006). We used only muscle tissue to avoid overestimation of residues in the digestive tract. We removed all the muscle tissue and dried it in an oven at 60 °C for 24 hours. The dried tissues were macerated until obtaining 1.0 g for each sample and were subjected to acid digestion to convert all forms of mercury into inorganic mercury (Hg^{+2}). For this, 10 mL of 55 % nitric acid (HNO_3) and 2 mL of 30 % hydrogen peroxide (H_2O_2) were added to each tissue sample in a reflux system for 1 hour at 60 °C and 5 hours at 100 °C (Campbell et al., 1986; Escobar-Sánchez, 2010).

The sediment samples were treated according to the protocol developed by IDEAM (2009) to analyse heavy metals in sediments. The samples were opened and dried at room temperature for 24 hours, and then in an oven at 60 °C for 12 hours. They were macerated until obtaining 4.0 g for each sample. They were subjected to acid digestion as above for 2 hours at 100 °C. Finally, both sediment and tissue samples were diluted with 25 ml of deionised water, filtered, and stored in plastic bottles at 0 °C. To determine the concentration of mercury (Hg^{+2}) in tissue and sediment samples, we follow Shaw et al. (1988), so we used cold vapour atomic absorption spectrophotometry (CVAAS) with sodium borohydride ($NaBH_4$) in the Laboratorio de Análisis Industriales of the Universidad del Valle (LAI), which is certified by national and international agencies regulating laboratory certification of high quality (Icontec, Icontec International, IQNet). Quality control samples included calibration curve and lab duplicates for each sample, without anomalies reported by the LAI.

Data analysis

The mercury bioaccumulation index, the ratio between tissue sample's metal concentration and the concentration in the corresponding sediment sample (Ahumada, 1994), was calculated for each frog sample. We assessed differences in mercury concentration between the tissue samples and sediment samples by applying a U-Mann Whitney test since parametric assumptions were not met for data (Shapiro-Wilk normality test: tissues W = 0.68085, p-value = 1.365×10^{-3}, and sediments W = 0.57804, p-value = 8.784×10^{-5}). We performed a Spearman correlation to determine the correspondence between mercury concentration in sediment and the tissues. Analyses were performed with the CAR package v.3.0-3 (Fox et al., 2021) in the R programming version 4.0.5 (R Core Team, 2021).

RESULTS

The streams sampled correspond to small water bodies that are tributaries of main rivers such as the Felidia and Pichindé rivers, which in turn are tributaries of the Cali River. We found an assemblage of amphibians composed of arboreal species and notably dominated by directly-developing frogs of the genus *Pristimantis* (family Strabomantidae).

Total mercury concentrations found in muscle tissues were lower (mean ± standard deviation, 0.0094 ± 0.0145) and less variable (range: 0.0004 - 0.0426 $\mu g.g^{-1}$) than those found in sediments (mean: 0.4917 ± 0.7829, range: 0.00532 and 2.2 $\mu g.g^{-1}$). The bioaccumulation indexes exhibited negative values (Table 1) since mercury concentrations in the muscle tissues were lower than in the sediment samples (Mann Whitney U = 57, p = 9.972×10^{-3}). However, alarming mercury concentrations were found in two sediment samples, one taken from a place where there was a mill for gold mining two years ago. The mercury concentration in sediments was not significantly associated with tissues, but the correlation coefficient was moderated and positive (Spearman's correlation $\rho = 0.55$, p = 0.154), probably due to the small sample size (n = 8).

DISCUSSION

The mercury concentration in muscle tissues of direct-developing frogs in the high elevation of Colombian Andes was lower than in strictly aquatic animals (Alvarez et al., 2012; Palacios-Torres et al., 2018), other anurans with larval stage in lower elevation sites affected by gold mining in Colombia (Hernandez-Cordoba et al., 2013), and the control treatment of controlled experiments with dietary mercury in wood frog tadpoles (Wada et al., 2011). Nevertheless, they were similar to the methylmercury concentration in the tissues of adult wood frogs in forested landscapes in the north-eastern US (Faccio et al., 2019). Besides, we found high mercury concentrations in sediment samples from streams in the study area, which are comparable to the concentrations of mercury found in sediments of areas with high impact of mining activity (Olivero et al., 1998; Hernandez-Cordoba et al., 2013). Differences in mercury concentrations between sediment samples and muscle tissues resulted in negative bioaccumulation indexes. Therefore, results suggest that direct-developing frogs are not exempt from contamination by heavy metals released into the environment; however, the bioaccumulation levels differ from frogs with aquatic larval stages due to the different life histories and behaviours that determine the exposure to contaminants.

Two sediment samples had high mercury concentrations (1.4 and 2.2 μg.g^{-1}) that exceed the risk threshold of 1.0749 μg.g^{-1} proposed by the World Health Organization (Betancur-Corredor et al., 2018) and are similar to samples from sites with a high impact of gold mining (Olivero et al., 1998; Hernandez-Cordoba et al., 2013). These samples came from places very close to mills that used mercury during the process of gold amalgamation (Palacios-Torres et al., 2018), and their concentrations are alarming since they were found in sediments of streams that are tributaries of Felidia, Pichindé, and Cali rivers, which provide untreated water to more than 7,000 people in the rural areas of Cali. It should be noted that for Colombia there is no guideline that estimates the maximum threshold allowed for mercury in sediments, however, other countries such as Canada, USA, Brazil estimate thresholds that do not exceed 0.35 μg.g^{-1} (Burton, 2002; CONAMA, 2004)

We found mercury in adult frogs of the genus *Pristimantis*, which have a reproductive mode by direct development (Crump, 1974; Duellman, 1992), although they are not in direct contact with the habitats where the mercury is discharged at any stage of their life; therefore, the bioaccumulation of mercury in tissues occurs indirectly. The low organism-environment interaction explains the lower concentration of mercury we found in muscle tissues than in sediments, resulting in negative bioaccumulation indexes for the different species. However, there is a marginally significant association between tissue and sediment samples, indicating that as mercury concentration increases in the environment, it is higher in animal tissues. Thus, although direct-developing species do not directly contact stream sediments, the mercury concentration in these samples represents the mercury used or remaining in the study sites environment. Species of *Pristimantis* may have obtained mercury from the prey they consume, and the biomagnification processes may occur in the study area since amphibians are at the base of the terrestrial food chain (Rimmer et al., 2009; Liu et al., 2020). Mercury can also be released into the environment as vapour at a lower percentage during the amalgamation process (Betancur-Corredor et al., 2018). Thus, the mercury bioaccumulated by frogs can be in part derived from vapours released from this process.

Another possible explanation for the low concentration of mercury we found in muscle tissues than in sediments is the nature of the mercury. The ratio of methylmercury to total mercury in tissues of frogs can be more than 80 %, depending on the species and season (Wang et al., 2005), while this ratio in sediments can be even lower than 1 % (Shi et al., 2005), thus having a huge amount of non-bioaccumulative inorganic mercury. Then, comparing mercury concentrations in tissues of frogs and sediments using total mercury measurements may be underestimating the actual values of forms of mercury that bioaccumulate as methylmercury. Thus, sediments do not correspond to an accurate point of comparison for measurements of mercury bioaccumulation in the tissues of frogs as has already been mentioned in previous works (Smalling et al., 2019; 2021), especially when using measurements of total mercury and frogs of direct development that present a low interaction with this source.

In conclusion, gold mining negatively affects ecosystems by indiscriminate deforestation and contamination of water sources with heavy metals (Campbell et al., 1986; Swenson et al., 2011; Esdaile and Chalker, 2018; Palacios-Torres et al., 2018). In the high elevations of the Colombian Andes, we found mercury accumulation in an assemblage dominated by direct-developing frogs of genus *Pristimantis*. The total mercury concentrations we recorded in frog tissues were lower than those reported from other localities with gold mining and larval-stage frogs (Hernandez-Cordoba et al., 2013) and controlled experiments with mercury diet in larval-stage frogs (Wada et al., 2011), but similar to the methylmercury levels found in the tissues of adult wood frogs (Faccio et al., 2019). The bioaccumulation index was negative, which can be related to the fact that we compared tissue samples obtained from direct-developing frogs with stream sediment samples, an aquatic environment with which these frogs do not have a close relationship. Also, another possible explanation is because the methylmercury is the form of mercury founded mainly in the tissues of frogs (Wang et al., 2005), which can present low levels in sediment samples (Shi et al., 2005), then the use of total mercury measurement comparing frog tissues and sediments may not correspond to an accurate way of comparison (Smalling et al., 2019; 2021).

Results suggest that the assemblage is indirectly accumulating heavy metals, probably by feeding insects with aquatic life-stages. The accumulation of mercury in animals that do not have direct contact with aquatic environments, where heavy metals are dumped due to gold mining, is a threat to the ecosystems' stability. In particular, the accumulation of heavy metals in frogs can produce a process of biomagnification since amphibians are at the base of the terrestrial food chain, which can threaten biodiversity due to the adverse effects of heavy-metals contamination on the survival and reproduction of wildlife (Rimmer et al., 2009; Hernandez-Cordoba et al., 2013; Liu et al., 2020).

Although our small sample size does not allow us to know the variability of mercury concentrations in the study site and makes it difficult to carry out statistical analyses, these results represent valuable information on little-known dynamics in high mountain ecosystems that are strongly intervened by actions of illegal mining in Colombia. This demonstrates the importance of control actions by environmental authorities in national parks to prevent this type of activity and, in this way, mitigate the harmful effects on the environment. In the same way, this work represents a starting point for future studies in this field in high mountain ecosystems. For future studies we recommend expanding in a gradient system that allows the comparison of species of direct-developing frogs with species with aquatic larval stages; consider different sources of mercury in the environment in addition to sediments such as waters and potential diet of the frog species examined; and not be limited to total mercury measurements but also measure methylmercury of the different samples.

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Authors' Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Oscar Mauricio Cuellar-Valencia and Gustavo Adolfo Rodriguez-Salazar. The first draft of the manuscript was written by Oscar Mauricio Cuellar-Valencia and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical Statement

This research was conducted under the authorisation of the National Authority of Environmental Licenses and the Ministry of Environment and Sustainable Development of Colombia (Resolución 1070 del 28 de Agosto de 2015), and Parques Nacionales Naturales de Colombia (aval No. 20172200004353 del 26 septiembre del 2017).

Data accessibility

The data that support the findings of this study are openly available in Figshare at https://figshare.com/s/8fa3463ef88a7ad20b1c.

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