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The Impacts of Mining in Andes: Water pollution and Metal Bioaccumulation in Amphibians at the Intag valley

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RESUMEN

El Valle de Intag, en los Andes ecuatorianos, es un hotspot de biodiversidad reconocido a nivel mundial que se encuentra amenazado por actividades de prospección minera de cobre. Este estudio proporciona la primera evaluación integrada de la calidad del agua y la bioacumulación de metales en anfibios durante una fase de exploración minera. Analizamos la química del agua en sitios impactados y de control, y la acumulación de metales en cuatro especies de ranas con distintas estrategias reproductivas.

Se detectaron concentraciones elevadas de aluminio, cobre, plata y zinc, que superaron los límites establecidos por la normativa ambiental ecuatoriana. Los análisis de bioacumulación mostraron diferencias interespecíficas significativas en las concentraciones de distintos metales, posiblemente relacionadas con las estrategias reproductivas, el uso del microhábitat de cada especie, y la influencia de rasgos fisiológicos o ecológicos. El modelado espacial confirmó que los patrones de dispersión de metales coinciden con las actividades previas de prospección minera.

Los resultados subrayan la vulnerabilidad de los anfibios ante la contaminación asociada a la minería y respaldan los esfuerzos de monitoreo y conservación liderados por las comunidades locales. Este novedoso estudio proporciona datos de referencia vitales para la regulación ambiental y evidencia el costo ecológico de la minería exploratoria en uno de los ecosistemas más biodiversos y sensibles del planeta.

Palabras clave: Minería de cobre, bioacumulación en anfibios, metales pesados, calidad del agua, especies bioindicadoras, ríos tropicales, Ecuador, Intag, conservación.

Abstract

The Intag Valley in the Ecuadorian Andes is a globally recognized biodiversity hotspot threatened by exploratory copper mining. This study provides the first integrated assessment of water quality and amphibian metal bioaccumulation during a mining exploration phase. We analyzed water chemistry across impacted and control sites, and metal accumulation in four frog species with distinct reproductive strategies.

Elevated levels of aluminum, copper, silver, and zinc were detected in water samples, exceeding Ecuadorian environmental thresholds. Bioaccumulation analyses showed significant interspecific differences in concentrations of several metals, possibly related to species' reproductive modes, microhabitat use, and possible influence of physiological or ecological traits. Spatial modeling verified that the metal dispersion patterns matched previous exploratory activities.

Results focus on the vulnerability of amphibians to mining related contamination and support community-led monitoring and conservation efforts. This novel study offers vital baseline data for the development of environmental regulation in the country and highlights the ecological cost of exploratory mining in one of the world's most sensitive and biodiverse ecosystems.

Key words: Copper mining, amphibian bioaccumulation, heavy metals, water quality, bioindicator species, Tropical streams, Ecuador, Intag, Conservation.

CONTENT TABLE

INTRODUCTION

The Intag Valley, in the Andes of Ecuador, is an internationally recognized biodiversity hotspot situated in the convergence of the Ecuadorian Chocó and the Tropical Andes. This region hosts many threatened species, including the spectacled bear (*Tremarctos ornatus*) and the jaguar (*Panthera onca*) (D'Amico, 2012; Torres-Jiménez et al., 2024), and endemic and endangered plants and animals such as the brown-headed spider monkey (*Ateles fusciceps fusciceps*), the longnose harlequin frog (*Atelopus longirostris*) and the recently discovered Intag's resistance rocket frog (Freile et al., 2020; Roy et al., 2018).

Local communities have been engaged in a long fight against mining activities and recently filed a legal action against the Ecuadorian Government to protect the biodiversity of the region (Lang, 2024). The proposed mining project Llurimagua poses a threat not only to biodiversity, water resources and primary forests, but also to the local economy and the well-being of the people who depend on the land. This project, which plans on developing an open-pit mine mainly targeting for copper and molybdenum, and encompasses an estimated area of almost 5000 hectares, is a joint venture between Ecuador's state-owned Empresa Nacional Minera (ENAMI EP) and the Chilean state-owned CODELCO (Avcı, 2017; Beltrán, 2020; Olmedo León, 2016; Tello Sánchez, 2016; Walter et al., 2016).

Mining activities have been associated with negative impacts on aquatic ecosystems and its biodiversity through water pollution, habitat destruction, and alterations in water chemistry (Gendron, 2013; Giam et al., 2018; Kimmel & Argent, 2019; Mayani-Parás et al., 2019; Sasaki et al., 2015; Simonin et al., 2021; Zocche et al., 2014). Previous research has demonstrated the importance of amphibians as indicator species for ecosystem health and water quality due to their vulnerability and sensitivity to environmental changes, as well as their role in nutrient cycling and maintaining the ecological balance (Chesnut, 1999; Fulton, 2013; Gendron, 2013; Homyack & Giuliano, 2023; Hutton et al., 2020; Mayani-Parás et al., 2019; Mazerolle, 2003; Sievers et al., 2018; Wozniak, 2001). Studies have also shown that mining activities have significant ecological impacts on amphibians, including decreased population size, deformities, and death due to habitat destruction, water pollution, and soil degradation (Adlassnig et al., 2013; do Amaral et al., 2019; Hintz & Relyea, 2019; Mazerolle, 2003; Meis, 1999; Pond et al., 2008; Price et al., 2016; REI Consultants, Inc. et al., 2004).

The most known case of mining activities in Intag is that of Junín, a small town south of Cotacachi-Cayapas National Park, in western García Moreno parish, Cotacachi canton, Imbabura province. Mining-related history in Junín initiated in the early 1980s, incentivized by the identification of significant mineral deposits, including copper, molybdenum, gold, and silver (Sacher & Chopard, 2017; Tello Sánchez, 2016). Bishimetal’s initial exploration in the 1990s, which included 30 perforations, released considerable amounts of toxic substances like arsenic (Kneas, 2016; Sacher & Chopard, 2017). Later, the Canadian company Ascendant Copper (later known as Copper Mesa) acquired concessions in the early 2000s, encountering strong opposition from local communities that ultimately led to its exit in 2008 (JICA-MMAJ, 1996; Sacher & Chopard, 2017; Tello Sánchez, 2016; Wahren & Schvartz, 2015). In 2011, ENAMI EP acquired mining rights and launched the Llurimagua project in partnership with CODELCO, leading to extensive exploration activities that included advanced drilling operations beginning in 2015 (Cardno, 2014, 2018). Although a total of 90 perforations were carried out as part of these exploration efforts, definitive information regarding their final locations is still unavailable, as in the case of the drills carried out by Bishimetal;

existing data only reflects initially proposed sites. The project has faced significant environmental and social challenges, as the project's environmental impact assessment (EIA) underestimated risks and unaddressed concerns about potential water contamination and habitat loss, raised. Moreover, in 2023, a provincial court ruled that ENAMI and CODELCO had violated both Nature and community rights, cancelling their licenses (Lang, 2024). Adding to the risk, the presence of pyrite and arsenic-bearing minerals in the region's geology poses a significant threat of acid mine drainage, which can lead to long-term water contamination (Tello Sánchez, 2016). Furthermore, abandoned mining sites from previous operations continue to contribute to water pollution and environmental degradation (Knee & Encalada, 2014; Zambrano-Romero et al., 2025). Crucially, independent monitoring efforts have documented significant water quality degradation, including increased levels of heavy metals, acidity, and electrical conductivity, negatively impacting the region's biodiversity (Acosta et al., 2020; Knee & Encalada, 2012; Murillo Martín, 2016; Roy et al., 2018; Sacher & Chopard, 2017; Tapia et al., 2017; Zorrilla et al., 2012). However, assessing the environmental impact of mining activities remains a significant challenge, as there is an important uncertainty surrounding the pristine state and crucial parameters of the ecosystem. This absence of pre-disturbance information and the precise locations of perforations makes it truly difficult to accurately quantify the degree of environmental degradation resulting from mining operations, including the effects of drilling activities.

In the realm of environmental challenges, mining activities stand out as one of the most significant threats to the balance of biodiversity (De Castro Pena et al., 2017; Martins-Oliveira et al., 2021; Murguía et al., 2016; Siqueira-Gay et al., 2020; Sonter et al., 2018; Van Dover, 2011; Virah-Sawmy et al., 2014). Ecuador, a global hotspot for biodiversity, finds itself at a critical juncture, especially because the effects of mining are largely understudied. In this context, the proposed research constitutes an essential first step in an area with a long tradition of resistance against mining. To date, no studies assessing water quality and heavy metal bioaccumulation in amphibians within an area undergoing mining exploration have been reported. This research, therefore, provides a novel scientific contribution by documenting the potential ecological impacts of mining in a highly biodiverse ecosystem prior to extraction activities.

This study evaluates mining contaminant impacts in the Intag Valley by comparing water quality between polluted and control sites and assessing metal accumulation in frog species with different reproductive strategies. Given the potential for metal contamination from exploratory mining, we hypothesize that water samples from mining-affected sites will have significantly higher metal levels than those from control sites. On the other hand, we expect frog species with tadpole stages, which are more exposed to aquatic environments, to accumulate higher concentrations of metals in their tissues compared to species with direct development.

METHODS

Study area

The study area is located within the Junín Communal Reserve, in Intag, Imbabura Province, Ecuador (Figure 1). The research specifically focused on the Junín River and La Fortuna Stream (Table 1), which are found in a native lower montane rain forest characterized by a dense vegetation cover with abundant trees, epiphytes, and bryophytes, frequent mist, approximately 1174.1 mm of rainfall, 85.2% relative humidity, and 10.4ºC (Cardno, 2018).

The Junín River has multiple branches, some of which are presumed to have healthy water (like the one with point J1), and a large section that in the past was visibly affected by mining activities (from J9A to J3, where it meets with J1's branch). The primary branch originates at point J9A and flows downstream to J3, where it converges with a secondary branch ending at J1 and continues further downstream, passing through J2. Particularly, the main branch also receives an additional small tributary (not visible in our river network layer) at point GM. At this confluence (GM), two waterfalls are present: one formed by the continuation of the main branch (with its last upstream point being J4), and the other originating from the aforementioned smaller tributary. Consequently, the main branch extends from J9A to J4, briefly forming a waterfall before continuing to GM, where it meets the tributary's waterfall and the river flows to J3. The studied river section, of a width that varies from 3 to 10 meters approximately, has three waterfalls in the upper section and one waterfall in the middle section. It is surrounded by vegetation, its substrate is mainly formed by sand, silt and rocks, and has several monitoring points for water quality established both previously and during the study period.

La Fortuna Stream, which has been entirely affected by the drillings of the past exploratory mining activities, is formed by two tributaries, one of them being the main one, that meet at points formerly named F1 and F3 and continue downstream. Hot water inputs coming from the drilling wells can be found in the higher section, as well as in the small branches where F1 and F3 are located, which were not monitored in this study. Following downstream, we find point F2, which was also not monitored here. In the present study, the confluence of tributaries F1 and F3 (named FB) and the confluence of a hot water input with the stream between FB and F2 (named FA) were monitored. It has an approximate width of 3 to 8 meters, is surrounded by vegetation and its substrate is mainly formed by silt and rocks.

Collection and processing of frogs samples

Two species with direct development (*Pristimantis w-nigrum* and *Pristimantis appendiculatus*), a species with a tadpole development (*Espadarana prosoblepon*), and a species with an atypical direct development (*Ectopoglossus* sp. nov.), were targeted. Prior to the field phase, *Ectopoglossus* sp. nov. was thought to have a typical tadpole development, but later information indicated that the species has an atypical direct development with a tadpole shape that does not develop in water, although its life cycle is still not completely well known (A. Terán-Valdez, personal communication, December 17, 2024). Five adult individuals from each species were collected (N = 20) between June 19th and June 22nd, 2024. Nocturnal surveys were conducted by four people between 7:00 PM and 12:00 AM, actively searching for target species within their respective habitats. All specimens and tissues were legally collected under research and collection permits issued by the Ministerio de Ambiente, Agua y Transición Ecológica (MAATE) of Ecuador (Permit No. MAATE-DNB-CM-2022-0245), under which ethical approvals for this study were obtained. Upon capture, liver, muscle, and skin tissue samples were excised from each individual using ceramic tools to minimize metal contamination. The samples were then dried with filter paper, weighed, and stored in Eppendorf tubes containing nitric acid. A minimum of 200 mg of dry tissue was homogenized from each sample to ensure sufficient material for metal analysis. Metal and metalloids concentrations were determined on acid digested muscle tissues using a ThermoScientific iCAP 7400 ICP-OES at Universidad de las Américas (UDLA-Ecuador). For every 10 samples, 2 blanks and 1 certified reference material fish tissue were analyzed to control the accuracy of the procedure. Average CRM recovery was 95%.

Water sampling design and methods

Microbiological analysis.

Sterile flasks were used to collect and refrigerate water samples, which were analyzed within 12 hours. One mL of each raw sample was plated on selective 3 M™ Petrifilm™ 6404/6414/6444 plates (3 M Microbiology Products, St. Paul, MN) and incubated following manufacturer guidelines. Total coliform and *Escherichia coli* concentrations were determined after 24–48 hours of incubation at 30 °C. Red or blue colonies with gas bubbles confirmed coliforms, while blue colonies with gas bubbles confirmed *E. coli*. Total coliform counts between 25 and 250 colonies per plate yield accurate results (Maurice et al., 2019).

Physico-chemical and metal analysis.

Physico-chemical characterization of water samples was conducted according to standardized protocols as previously published (Borja-Serrano et al., 2020; Vinueza et al., 2021). Dissolved oxygen (DO), temperature (SM 4500-O A), electrical conductivity (SM 2510), and pH (SM 4500 H+) were measured in situ with a YSI Professional Plus hand multi-parameter according to the manufacturer's instructions (Ohio, United States). Alkalinity (SM 2320 B), ammonium (Salicilate Method HACH 8155), chemical oxygen demand (SM 5220 D), chloride (HACH 8113), fluoride (USEPA 10225), hardness (HACH 8030), nitrite (HACH 8507), nitrate (HACH 8507), phosphate (SM 4500-P-E), sulfate (USEPA 375.4; SM4500-SO42-E), total solids (SM 2540 B), and turbidity (ISO 7027) were analyzed in the AQUA-BIO Laboratory at Universidad San Francisco de Quito.

Metal analysis (27 metals and 5 metalloids) on filtered (0.45 μm) and acidified water (2% HNO_3) samples was conducted at Universidad de las Américas (UDLA-Ecuador) using a ThermoScientific iCAP 7400 ICP-OES. Commercial standards in dilute nitric acid (ICP multi-element standard solution IV 23 elements LOT: HC31118355) were used to prepare standard solutions. Detection (LOD) and quantification (LOQ) limits were calculated by analyzing blank samples with at least 8 replicates, adding the average of the blank values with 3 and 10-fold the standard deviation, respectively.

Quality assurance and control.

All measurements were made by duplicates. Quality Control in metal analysis was conducted employing CRM ERA 500 – WatRTM Pollution Trace metals, in which all metals were measured every 10 samples. Recovery percentages were calculated to determine matrix effects and measurement accurateness. The recoveries varied between 99.7% and 100%.

The quality of the water was established under the admissible levels proposed by the Ecuadorian Environment Ministry's legislation under the quality criteria for the preservation of aquatic life and wildlife in freshwater, marine and estuarine waters (MAE, 2015).

Statistical analysis

Regarding the data and variables analyses were performed in RStudio yielding a 95% confidence interval.

Given the proximity and non-independence of sampling sites J9A and J9B, the data from both sites were averaged to create a single representative point, J9.

Inter-species and reproductive strategies comparisons of metal(loid) concentrations from our interest (Al, Ba, Cu, Fe, Mn, Na, Si, Sr, and Zn) and body size (i.e., snout-vent lengths) were performed using appropriate statistical tests (Kruskal-Wallis tests and Wilcoxon rank-sum tests) based on sample size and data normality.

Correlations were conducted to assess the relationships between main metal(loid) concentrations in frog tissues, water parameters, and main metal(loid) levels across sampling locations. Spearman correlations were employed for most analyses, although Pearson correlations were used when they suited better due to data characteristics.

Spatial modeling of metal distribution in surface waters

Modeling of spatial distribution of key metals (aluminum, copper, iron, lithium, and manganese) was performed within the study area using ArcGIS Pro and diverse layers:

- Hydrography: A polyline layer representing the river network of Ecuador.
- Elevation: A Digital Elevation Model (DEM) raster layer sourced from the Advanced Land Observing Satellite Phased Array Type L-band Synthetic Aperture Radar (ALOS PALSAR) 12.5 m.
- Sampling point data: A point feature layer containing the locations (X, Y coordinates) of the water sampling sites (as detailed in Table 1), with attributes for each point (except J9) including the measured concentrations of the selected metals.

Prior to spatial modeling, an exploratory spatial data analysis (histograms, trend analyses, and quantile-quantile plots) was effectuated for each metal concentration to understand its spatial characteristics. Semivariograms and covariance functions were examined to quantify spatial dependence and the range over which metal concentrations are correlated, and sensitivity analysis was conducted on semivariogram parameters (nugget, sill, and range) to optimize model fitting.

Although other spatial interpolation methods like Inverse Distance Weighting (IDW) and Cokriging were tested, Ordinary Kriging (OK) was selected as the main technique to create continuous surface maps of metal concentrations, as it demonstrated better predictive performance.

To estimate the accuracy and bias of prediction, and the overall performance of each Ordinary Kriging model, cross-validation was thoroughly evaluated, employing statistical metrics: Mean Error (ME), Root Mean Square Error (RMSE), Mean Standardized Error (MSE), Root Mean Square Standardized Error (RMSSE), and Average Standard Error (ASE). Results of the cross-validation analysis were used to refine the OK models, ensuring that resulting maps provide a visual representation of the spatial distribution of metals and potential contamination hotspots in the study area.

RESULTS

Metal analyses of water from the Junín River and La Fortuna Stream and amphibian tissues from the study area revealed the presence of several metals and metalloids (Table 2).

Bioaccumulation of metals in amphibians

Analyses of frog tissues revealed significant interspecific differences in barium ($H = 12.829$, $p = 0.005$), copper ($H = 14.874$, $p = 0.002$) and iron ($H = 8.0514$, $p = 0.045$) (Figure 2, a-c). *Ectopoglossus* sp. nov. exhibited the highest median Ba concentrations, significantly exceeding *Espadarana prosoblepon* and *Pristimantis w-nigrum* (pairwise $p = 0.048$ for both). *Ectopoglossus* sp. nov. had markedly higher Cu concentrations (median ~70 mg/kg), than *E. prosoblepon* (~1 mg/kg), *P. appendiculatus* (~6 mg/kg), and *P. w-nigrum* (~3 mg/kg) (pairwise $p = 0.048$ for all). *P. appendiculatus* also accumulated significantly more Cu than *E. prosoblepon* ($p = 0.048$). Conversely, *E. prosoblepon* had the highest Fe concentration (~37 mg/kg), significantly exceeding *Ectopoglossus* sp. nov. (median ~11 mg/kg) (pairwise $p = 0.048$).

Considering reproductive strategy, statistically significant differences in zinc levels were observed (Figure 2d), in addition to significant variations in barium ($H = 11.446$, $p = 0.003$), copper ($H = 14.143$, $p < 0.001$), and iron ($H = 7.8686$, $p = 0.02$) that were also evident when grouping by reproductive mode. While Kruskal-Wallis test identified significant zinc variation across strategies ($H = 7.0886$, $p = 0.03$), subsequent pairwise comparisons failed to detect significant differences (tadpole vs. typical direct development: $p = 0.058$). Similar to interspecific patterns, pairwise comparisons showed differences in Ba between the atypical direct development and both tadpole ($p = 0.016$) and typical direct development ($p = 0.008$). As for Cu concentrations, there were significant differences between atypical direct development and both tadpole ($p = 0.016$) and typical direct development ($p = 0.002$), and between tadpole and direct development ($p = 0.016$). Finally, Fe concentrations significantly differed between tadpole and atypical direct developments ($p = 0.024$).

While other metals and metalloids did not show significant interspecific differences (Figure 1 from Supplementary information), trace silver was detected in one *Ectopoglossus* sp. nov. individual, and mercury in two *Pristimantis w-nigrum* specimens collected close to La Fortuna Stream, suggesting potential localized contamination. Aluminum concentrations exhibited a wide range across all species, with *E. prosoblepon* showing the lowest median concentration, although considerable overlap existed between species. Lithium levels were generally low across species, with *Ectopoglossus* sp. nov. displaying the highest median and one individual of *P. w-nigrum* having a higher concentration, although the ranges overlapped considerably. Manganese concentrations were detected in all species, with *P. appendiculatus* exhibiting the highest median. Although silicon concentrations showed similar distributions across the four species and there was no clear visual separation between groups, one individual of *P. appendiculatus* had a notably higher concentration. Strontium was detected in all species, with *Ectopoglossus sp. nov.* showing a comparatively higher median concentration and wider data distribution. Finally, zinc concentrations were present in all species, with *P. w-nigrum* displaying the highest median. Therefore, *Ectopoglossus* sp. nov. mainly distinguished itself when accumulating Ba, Cu, and to a lesser extent Sr. These observations, while not statistically significant in this dataset, suggest some similarities in accumulation patterns between species, provide preliminary insights into the potential accumulation patterns of these elements in these amphibian species, and highlight the need for further investigation with a larger sample size.

However, interspecific differences in metal bioaccumulation raised concerns about the potential influence of frog size. To address this, frog sizes were analyzed per species, revealing that *Pristimantis* individuals tend to be bigger than *E. prosoblepon* and *Ectopoglossus* sp. nov. ($H = 15.011$, $p = 0.002$), with significant size differences between both *Pristimantis* species and the other two ($p = 0.048$ for all comparisons).

Correlation analyses revealed several significant relationships ($p < 0.05$) between metal concentrations in frog tissues and water quality parameters. Larger individuals were likely found near zones with elevated copper, lower atmospheric pressures, and reduced phosphorus, chloride, potassium, and turbidity. Moreover, high significant correlations ($p < 0.001$) between specific metals in amphibian tissues were revealed, indicating these may originate from the same environmental sources or co-accumulate via similar physiological or ecological processes.

Due to a lack of frog tissue metal concentration standards and criteria, our findings were compared with research from regions affected by gold or coal mining in Colombia (Cuellar-Valencia et al., 2023), Thailand (Intamat et al., 2016), and Brazil (Zocche et al., 2013) (Table 3). *Ectopoglossus* sp. nov.'s Cu concentrations (70.53 ± 52.8 mg/kg), though seven times lower than extreme values from coal-mined *Boana faber*'s liver (506 ± 63.25 mg/kg), surpassed levels in *Hoplobatrachus rugulosus* from a gold mining area by almost 3 times (25.2 ± 19.42 mg/kg), notable given the exploratory conditions prior to full-scale mining. Although copper levels from the other studied species remained much lower (means from 1.21 to 5.97 mg/kg), they were similar to those seen in muscle from coal-mined *Boana faber* (3.7 ± 5.69). *Espadarana prosoblepon*'s Fe levels (37.29 ± 11.6 mg/kg) were comparable to *H. rugulosus* from gold mined regions (44.35 ± 25.63 mg/kg) but more than 127 times lower than in *B. faber* from coal mining areas (4747 ± 215.06 mg/kg). Mercury was below detection limits in all our species except *Pristimantis w-*

nigrum (0.2265 ± 0.163 mg/kg), although levels were much higher than those reported in muscle from species of Colombian illegal gold mining sites: more than 66 times higher than *Pristimantis* sp. (0.0034 ± 0.0025 mg/kg), 10 times higher than *Pristimantis buckleyi* (0.0216 ± 0.019), and more than 566 times higher than *Pristimantis aff. calcaratus* (0.0004 ± 0.00). Mn concentrations from studied species were relatively low compared to coal and gold mining contexts, which showed levels even up to almost 45 times higher. Finally, Zn concentrations found in Intag's frogs, like *P. w-nigrum* (4.17 ± 2.5), were sometimes similar to those from gold mined areas, like *M. pulchra* (4.36 ± 3.00). However, all reported zinc levels in frogs from Intag remained more than 10 times lower than those from frogs in coal mines.

Water quality

Wata data revealed that some variables did not fulfill Ecuadorian quality standards, although comparisons were limited by undefined limits for several parameters. Regarding *in situ* parameters, measurements showed low pH values at most of the sampling points, including at control sites J1 and J9, while admissible values were met in FA, J4, and J5 (Figure 3). Conversely, COD, dissolved oxygen, nitrite, nitrate, As, Ba, Fe, and Mn levels met the standards across all sites.

Microbiological indicators revealed different patterns of contamination across sites. *E. coli* was only detected at J3 (100 CFU/100 mL), likely reflecting a transient or residual contamination event such as recent defecation by wildlife or due to the possible presence of livestock upstream. However, this value remained below the permitted level for surface water partial-body contact by the United States Environmental Protection Agency (USEPA, 2012). Total coliform counts showed higher levels at impacted sites FB (2500

CFU/100 mL) and FA (300 CFU/100 mL), surpassing the permitted level for surface water partial-body contact by the United States Environmental Protection Agency (USEPA, 2012). The presence of coliforms in La Fortuna Stream (points FA and FB) could be due to the possible presence of livestock or small houses in the upper part of the stream, although further studies should focus on confirming the source. Other impacted sites (J2, J3, J4) and control sites (J5 and J9) had lower levels (100 CFU/100 mL), suggesting some degree of diffuse or upstream contamination. Findings highlight the importance of carefully interpreting microbiological indicators in natural systems, as random contamination events could occur apart from anthropogenic impacts.

Water metal analyses reported a silver concentration of 0.038 mg/L at FA, almost four times Ecuador's limit (Figure 4a). As FA corresponds to the confluence of a hot water input and La Fortuna Stream (where the sample was taken), this suggests the possibility of even higher silver levels in the input itself, indicating that these inputs carry high concentrations into the stream.

Aluminum concentrations exceeded Ecuadorian limits at seven of the nine sites (Figure 4b). At FA, J4, and J5 (pH > 6.5), aluminum should have been below 0.1 mg/L, but FA exceeded this. Conversely, FB, GM, J1, J2, J3, and J9 also significantly exceeded Ecuador's aluminum limit of 0.005 mg/L, with FB being the most concerning at 0.878 mg/L, more than 175 times the admissible level.

Furthermore, high Cu and Zn concentrations exceeding Ecuadorian legal limits were also measured. Whereas Zn concentrations surpassing Ecuadorian legal limit (0.03 mg/L) were only detected in GM (0.399 mg/L, over 13 times the admissible concentration)

(Figure 4d), Cu should be below 0.005 mg/L, a criterion not met at any location except J4 (Figure 4c), with FB being the most concerning (0.341 mg/L, over 68 times the limit for the preservation of aquatic life and wildlife).

While some parameters do not have a limit established by the Ecuadorian legislation or measured values remained below limits (Table 1 from Supplementary information), atmospheric pressure and nitrites remained similar across most sampling points. COD values were measured at J2 (4.01 mg/L) and J9 (3.13 mg/L), while nitrates were only found in J1 (2.66 mg/L) and J2 (3.1 mg/L). Regarding phosphate and fluoride, the highest values were found in FA, while the lowest ones were those from FB. Although just minor differences were observed across points for Ca, K, Mg, Na, P, S, Li, and Si concentrations (Table 2 from Supplementary information), FA notably exhibited the highest Ca, Na, S, and Si levels, and J1 slightly differed from the other points in Mg concentration.

Compared to mining sites in other countries and within Ecuador (Table 4), copper concentrations in our study averaged higher levels than those reported in uranium mining (0.021 mg/L) and gold mining areas from Thailand, Romania, and Ecuador (except for two dry season measurements in 1998-99). Iron values from few sites were substantially lower than those from uranium and gold mining areas, as well as in mountaintop surface mines. Arsenic concentrations from two sites (J5 and FA) were higher than in uranium mined areas, but lower than Thailand's gold mined area. Manganese in the Intag Valley averaged significantly lower than in uranium and gold mined areas but comparable to mountaintop surface mines. Some Intag aluminum levels were similar to mountaintop mines from the United States of America and Suriname's gold mined area, with FB's concentration being markedly higher; however, Intag's concentrations remained below those at uranium mines from Portugal. Although Intag's Sr concentrations were lower than those at Portugal's uranium mines, Ba was higher. Zinc in water at GM reached 0.399 mg/L, exceeding levels in gold mining areas and such those from uranium mines.

Compared to other mining contexts (Table 5) in Suriname (Mol & Ouboter, 2004) and the United States of America (Muncy et al., 2014; Pond et al., 2008; Simonin et al., 2021; Wood & Williams, 2013), electrical conductivity in Junín was similar or even higher than in a gold mined area but lower than coal and mountaintop mines. Measured dissolved oxygen was higher than gold mines but a bit lower than coal mined areas. Regarding pH, values were lower than in coal mined areas, similar to some mountaintop mined areas, and higher than in gold mined areas. Water temperatures were lower than gold but higher than coal and mountaintop mined zones. On the other hand, measured total dissolved solids (TDS) and sulfates were similar to or higher than gold mined areas, but lower than mountaintop mines. Alkalinity was higher than in gold mined areas, although FB presented a lower value than those sites. However, turbidity, total hardness and chloride values from Intag were lower than those from gold mined areas.

Statistical analysis revealed a total of 65 significant ($p < 0.05$) and strong correlations (absolute correlation coefficient > 0.7) among variables, suggesting interdependencies and shared factors influencing the quality of study's area water. Therefore, the results indicated potentially distinct characteristics between the La Fortuna Stream and Junín

River sites, as FA and FB exhibited clear differences regarding some parameters and metal(loid)s. Moreover, the GM and J1 data could also indicate slight differences from the other sites, highlighting subtle site-specific variability. Finally, the data suggest that J1 differs from the other control sites and is more similar to the affected locations, although further studies are needed to assess whether all sampling sites are truly affected by drilling or if the measurements are due to natural causes.

Spatial modeling of metal distribution in surface waters

Similar spatial distribution patterns were observed across the modeled metals (Al, Cu, Fe, Li, and Mn) (Figure 5). Although exact locations of drilling wells are unknown, community explorations indicate their prevalence in the upper sections of both the Junín River and La Fortuna Stream, generally coinciding with preliminary locations. Modeled upstream higher metal concentrations supported the hypothesis that drilling introduces significant metal(loid)s loads, which eventually disperse as water flows downstream. In spite of this, models also revealed an increase in the concentrations of certain metals near GM, suggesting a possible localized contamination source explaining elevated concentrations observed downstream (J2 and J3). This was supported by historical community accounts and photographic evidence documenting past metal contamination from the smaller tributary at the GM site (described in the methodology section). This evidence included a persistent brown rock discoloration contrasting with the clearer appearance of the main tributary.

Higher Fe and Mn modeled concentrations in the J4 zone compared to J5 suggest possible existence of heavy metal inputs originating from drilling holes between both points. The higher concentrations of Mn and Cu in the lower reaches (sampling points J1-J3) than at

GM could be attributed to a possible metal source situated between these areas or, contrary to initial assumptions of its pristine condition, an unexpected contamination of J1's tributary. However, more data from the upper and middle reaches of this specific river branch would be required to confirm this, given J1's closeness to the confluence with the main tributary.

DISCUSSION

This study provides valuable preliminary evidence that even early exploratory phases of copper mining can result in significant environmental disturbances, with measurable effects on both water quality and amphibians. Despite the limited spatial and temporal scope of mining activities in the Intag Valley, and the small number of samples collected, findings suggest metal(loid) contaminants may be entering the freshwater system, threatening fauna.

Interspecific variation in metal(loid) accumulation likely reflects differences in physiology, habitat, and reproduction. For instance, high Cu in the waterfall-associated *Ectopoglossus* sp. nov. suggests aquatic exposure strongly influences bioaccumulation, even in this early mining phase. In contrast, *Espadarana prosoblepon*, a species with tadpole development and high-water contact, showed elevated Fe concentrations and low Cu, reinforcing concerns about low-level contamination (Azizishirazi et al., 2021). *Pristimantis* species (direct development, less aquatic), accumulated intermediate to high levels of Ba, Fe, and Zn, although with considerable variability. These contrasting patterns imply specific metal absorption mechanisms, variability of microhabitat, and physiological tolerances shaping bioaccumulation profiles. This emphasizes considering not just life history traits, but also habitat microconditions, trophic interactions, and exposure pathways, natural geochemical background, and potential amphibian osmoregulatory limitations, especially in environments with high electrical conductivity and dissolved ion concentrations (Hutton et al., 2020; Muncy et al., 2014; Schorr et al., 2013). Further research is needed on species-specific tolerances to naturally occurring or mining-enhanced metal concentrations present in their local habitats.

The trace concentration of silver detected in one *Ectopoglossus* sp. nov. individual together with high Cu levels question this species' physiological sensitivity and exposure routes, given its strong association with humid streamside/waterfall microenvironments potentially offering constant contact with metal-rich surface flows (including from past mining). While findings suggest this species could be a bioindicator for early-stage Cu and potentially Ag contamination, further research is needed to assess its reliability and specificity. Consequently, the endemic *Ectopoglossus* sp. nov. requires an immediate species-specific conservation plan and research into its life history, tolerance, and possible bioaccumulation pathways.

The presence of mercury, not typically associated with copper mining, in two *P. w-nigrum* individuals could indicate environment contamination, atmospheric deposition, or an unrecognized input. Indeed, while our results show certain patterns and interesting findings that suggest something is happening in the ecosystem, the precise origin of all detected contaminants, including mercury, cannot be completely attributed solely to the exploratory drilling phase without further investigation into other potential sources, emphasizing the need for amplified monitoring and broader toxicological evaluations. As amphibians are relatively long-lived, ecological impacts of metal exposure may take years to manifest, especially in terms of reproductive success and larval survival (Wood & Williams, 2013).

Compared to established gold and coal mining sites, Intag amphibian metal levels are significant considering the early mining stage. While not as extreme as those observed in coal mining, Cu levels in *Ectopoglossus* are particularly notable, suggesting that even preliminary exploration can result in metal exposure levels comparable to more developed activities. Zinc and manganese levels were generally lower in Intag species, possibly due to the lower intensity and shorter duration of mining activities. However, the single detection of Zn exceeding water thresholds still warns risk, pointing up the importance of early-stage monitoring and careful mitigation.

Observed correlations suggest amphibian body size may relate to environmental quality, potentially influenced by water quality gradients affecting growth or survival, indicating a need to include body measurements such as anuran body weight in future studies to better understand these relationships.

The heterogeneity in water metal concentrations across sampling sites suggests that metal release is spatially variable, likely influenced by hydrology, geology, and grade of affectation from past disturbance. Water quality standards exceeded for Al, Cu, Zn, and Ag at affected sites suggest exploratory mining can degrade water quality, with possible additional contributions from drilling-induced hydrothermal vents. Necessarily, all the exact locations of water entry points from drilling activities into the rivers in the area are not known, meaning that sudden physicochemical changes or contaminant spikes between sampling sites could potentially be attributed to these unmapped inputs, constituting a long-term contamination source even in the absence of ongoing mining (Brosse et al., 2011). Issues at some "healthy" sites also question current classification methods and point to the need for assessment frameworks that combine both dynamics and ecology.

Furthermore, it is essential to acknowledge that mining activities in Intag have historically been irregular and often lacked thorough environmental supervision, resulting in a complete absence of a true pre-drilling baseline for water quality and ecosystem health. The lack of baseline data makes it difficult to attribute the observed changes exclusively to current exploratory activities and highlights environmental management shortcomings. This situation also acts as an important observation for the Ministry of Environment, Water and Ecological Transition (MAATE), highlighting the urgent need for comprehensive and compulsory environmental impact assessments prior to, during, and following any extractive-related activities. Additionally, the loss of riparian buffers could also contribute to the loss of biodiversity in ecosystems like this one (Ríos-Touma et al., 2022). Therefore, future studies should increase the number of sampling points, particularly downstream, and incorporate truly undisturbed control sites with comparable ecological characteristics, ideally establishing a paired-watershed approach with a geologically and ecologically similar but unimpacted area to provide a stronger comparison.

Interpretation is further challenged by the absence of standardized thresholds for metal concentrations in amphibian tissues and the lack of a globally accepted Water Quality Index (WQI) integrating chemical and biological data, especially amphibians (Chidiac et al., 2023; Terneus Jácome & Yanez-Moretta, 2018). Developing scientifically practical WQIs and pollution indices, adaptable regionally but globally connected, is essential (Khadija et al., 2021), particularly for biodiverse nations like Ecuador, where freshwater systems host high biodiversity but lack regulations that integrate amphibians as bioindicators, as they tend to focus on fish and macroinvertebrates (Barraza et al., 2018; Feio et al., 2021; Ríos-Touma et al., 2014; Torremorell et al., 2021; Wingfield et al., 2021). It is essential to set precise toxicological thresholds for a broader range of variables in water and amphibian tissues, and to consider that these limits may require a more sophisticated approach, possibly using a tiered or categorical system of impact levels, or more gradual scales of effect, instead of just binary pass/fail standards. Therefore, legislation should evolve to include amphibians and deal with emerging stressors, including metal contamination. Future research should incorporate factors like BAF, morphology, size, seasonality, diet, and metal routes (Smalling et al., 2021; Stolyar et al., 2008; Zhelev et al., 2020; Zocche et al., 2013). Although not possible in the present study due to the small size of studied frogs, future research should avoid mixing tissues from different organs, as this would be ideal to differentiate how metals are accumulated in the different tissues. Also, including more individuals and a broader variety of species with different reproductive strategies, as well as specifically investigating larval stages which are often more sensitive to contaminants, would allow for a greater understanding of the patterns of bioaccumulation and the role of life history in exposure.

The conservation of amphibians demands protection for both riverine habitats and surrounding land areas, as they need both environments for reproduction and survival (Vojar, 2006). Legal frameworks should establish biodiversity protection over mining extractions, especially in regions with ecologically sensitive environments. Although in Ecuador Nature holds constitutional rights, these are increasingly threatened by extractive projects, as in the case of the Los Cedros Biological Reserve (Guayasamin et al., 2021; Hutter & Guayasamin, 2015). For that reason, systematic inclusion of mining as a driver of extinction risk is necessary in conservation assessments (Mayani-Parás et al., 2019). This is not only limited to habitat loss but also contamination of aquatic systems and indirect effects on species distributions (De Castro Pena et al., 2017; Sonter et al., 2020).

Future research should combine long-term monitoring with investigation of population viability and reproductive success, specifically in relation to exposure to harmful substances and threatened species like the *Atelopus* (La Marca et al., 2005). Additionally, negative correlations between anuran richness and metal pollution suggest that amphibian diversity is declining due to sustained contamination (Ficken & Byrne, 2013).

Beyond the ecological impacts on amphibians, the observed water quality degradation, specifically elevated aluminum, copper, zinc, and silver, raises potential human health concerns. Communities relying on these rivers for drinking, cooking, or irrigation may be exposed to these contaminants, which are associated with various adverse health effects, such as gastrointestinal problems or damage of immune function (Badeenezhad et al., 2023; Kumar et al., 2022). The detection of mercury in amphibians is especially alarming given its potent neurotoxicity and capacity for bioaccumulation in food chains. As this study indicates, metal(loid)s may be entering freshwater systems even during early exploratory mining, creating a potential route for human exposure and highlighting the critical necessity for careful monitoring of drinking water sources along with proper health risk evaluations for local communities.

While this study is preliminary and further data are needed for a more comprehensive understanding, it represents a novel and critical first step in assessing the impact of early-stage mining activities on amphibians and freshwater ecosystems. The findings provide important insights into the potential for metal contamination even in exploratory mining phases, offering a baseline for future research in this region. Nonetheless, it is vital to treat these preliminary data and their analyses cautiously, taking into account the study's limitations, including the limited sample sizes and the possible confounding effects of natural habitat characteristics compared to drilling-related impacts. Although findings do not definitively clarify the extent to which the ongoing exploratory phase affects the ecosystem, they do highlight specific trends and significant insights that suggest ecological changes are happening, whilst the exact causes and processes behind these shifts have yet to be fully understood.

In sum, this baseline research demonstrates potential ecological risks from early exploratory mining in the Intag Valley, detectable in water and amphibian tissues. Observed accumulation patterns reflect how habitat, reproduction, and physiology influence exposure, demonstrating complex ecological responses and the need for site-specific monitoring and broader regulatory reform. Future studies should incorporate more sampling points, a broader selection of species with different reproductive strategies, and increased individual sampling, potentially considering the weight of the frogs, distinct larval phases, and the influence of seasonality. In conclusion, these findings focus attention on the urgency of ceasing future mining developments in the Intag Valley, as they provide powerful evidence to support conservation and community sustainable actions designed on protecting this unique ecosystem.

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TABLES

Table 1. Coordinates of the points determined for water samples. Latitude and longitude are expressed in decimal degrees, and elevation is given in meters above sea level.

Sampling point	Latitude	Longitude	Elevation (m.a.s.l.)	Observations
FA	0.309952	-78.650726	1831	Confluence of a hot water input from perforations with the stream. Affected site
FB	0.310302	-78.650344	1844	Affected site
GM	0.311889	-78.657227	1755	Affected site
J1	0.311570	-78.659070	1707	Control site
J2	0.310809	-78.658685	1698	Affected site
J3	0.311549	-78.658957	1707	Affected site
J4	0.312015	-78.657181	1761	Affected site
J5	0.314299	-78.655980	1858	Control site
J9A	0.314895	-78.656057	1889	Control site
J9B	0.314795	-78.656019	1882	Control site
J9				No real location, data averaged from J9A and J9B. Control site

Table 2. Descriptive statistical parameters (mean ± standard deviation) of detected metal(loid) concentration (in milligrams per liter) in all water sampling points and tissues (in milligrams per kilogram) of frog species studied, as well as the maximum concentration allowed by the Ecuadorian legislation for the preservation of aquatic life and wildlife in freshwater, marine and estuarine waters (MAE, 2015).

Element	**Water (concentrations expressed in mg/L)**		**Mix of liver, muscle and skin (concentrations expressed in mg/kg)**			
	Ecuadorian legislation	This study (N = 9)	*Ectopoglossus* sp. nov. (N = 5)	*Espadarana prosoblepon* (N = 5)	*Pristimantis appendiculatus* (N = 5)	*Pristimantis w-nigrum* (N = 5)
Ca		10.40 ± 19.7	416.6 ± 129.0	347.3 ± 41.9	370 ± 43.6	334.4 ± 60.3
K		0.59 ± 0.2	1583 ± 219.8	1172 ± 237.8	2075 ± 149.8	1706 ± 1136
Mg		0.41 ± 0.4	139.6 ± 8.2	122.8 ± 14.9	174.3 ± 11.7	160.3 ± 77.8
Na		3.05 ± 4.8	373.4 ± 40.4	452.4 ± 58.0	508.2 ± 55.8	405.9 ± 181.6
P		0.15 ± 0.1	1339 ± 239.1	1030 ± 208.7	1893 ± 225.8	1664 ± 1052
S		11.38 ± 20.4	816.9 ± 103.6	651.8 ± 118.2	1139 ± 134.7	1095 ± 690.1

Ag	0.01	0.038*	1.031*	BLD	BLD	BLD
Al	0.1 (1)	0.18 ± 0.3	16.79 ± 10.4	11.96 ± 3.0	23.03 ± 15.7	20.23 ± 10.6
As	0.05	0.034 ± 0.007	BLD	BLD	BLD	BLD
Ba	1	0.04 ± 0.01	3.52 ± 0.6[bd]	2.09 ± 0.2[a]	2.62 ± 0.5	2.26 ± 0.4[a]
Cu	0.005	0.17 ± 0.09	70.53 ± 52.8[bcd]	1.21 ± 0.05[ac]	5.97 ± 4.8[ab]	2.88 ± 1.4[a]
Fe	0.3	0.0395 ± 0.03	11.43 ± 7.7[b]	37.29 ± 11.6[a]	18.93 ± 11.1	22.73 ± 15.1
Hg	0.0002	BLD	BLD	BLD	BLD	0.2265 ± 0.163
Li		0.03 ± 0.02	0.607 ± 0.13	0.3087 ± 0.19	0.242 ± 0.2	0.39 ± 0.3
Mn	0.1	0.0314 ± 0.007	0.55 ± 0.03	0.44 ± 0.3	0.91 ± 0.2	0.53 ± 0.3
Si		5.45 ± 1.7	5.28 ± 2.0	4.35 ± 1.4	5.15 ± 3.8	4.07 ± 1.1
Sr		0.16 ± 0.3	1.61 ± 0.8	1.07 ± 0.1	1.21 ± 0.4	0.97 ± 0.15
Zn	0.03	0.3985*	2.58 ± 0.6	2.19 ± 0.5	3.44 ± 0.6	4.17 ± 2.5

BLD below the limit of detection

Values in red exceed the maximum concentration allowed by the Ecuadorian legislation

* Indicates that the standard deviation was not calculated due to single data

(1) If pH is lower than 6.5, the limit is 0.005 mg/L

Significant differences ($p < 0.05$) are marked with the letters a, b, c, and d, which indicate differences among species

[a] is *Ectopoglossus* sp. nov.

[b] is *Espadarana prosoblepon*

[c] is *Pristimantis appendiculatus*

[d] is *Pristimantis w-nigrum*

Table 3. Data comparison of mean metal concentrations (mg/kg) in amphibian tissues between this study (exploratory copper mining phase) and other studies conducted in gold (Cuellar-Valencia et al., 2023; Intamat et al., 2016) and coal mining (Zocche et al., 2013) contexts. Species studied and origin of samples are indicated. BLD = Below Limit of Detection.

Species / Museum num.	**Tissue**	**Metal (mg/kg)**						**Reference**
		Al	**Cu**	**Fe**	**Hg**	**Mn**	**Zn**	
Ectopoglossus sp. nov. (N = 5) / CJ 15014-15018	Mix of liver, muscle and skin	16.79 ± 10.4	70.53 ± 52.8	11.43 ± 7.7	BLD	0.55 ± 0.03	2.58 ± 0.6	This study
Espadarana prosoblepon (N =		11.96 ± 3.0	1.21 ± 0.05	37.29 ± 11.6	BLD	0.44 ± 0.3	2.19 ± 0.5	This study

5) / CJ 15009-15013								
Pristimantis appendiculatus (N = 5) / CJ 15000-15003, 15019		23.03 ± 15.7	5.97 ± 4.8	18.93 ± 11.1	BLD	0.91 ± 0.2	3.44 ± 0.6	This study
Pristimantis w-nigrum (N = 5) / CJ 15004-15008		20.23 ± 10.6	2.88 ± 1.4	22.73 ± 15.1	0.2265 ± 0.163	0.53 ± 0.3	4.17 ± 2.5	This study
Pristimantis sp. (N = 2)	Muscle	-	-	-	0.0034 ± 0.0025	-	-	Cuellar-Valencia et al. (2023)
Pristimantis brevifrons (N =1)		-	-	-	0.0027 ± NA	-	-	
Pristimantis buckleyi (N = 3)		-	-	-	0.0216 ± 0.019	-	-	
Pristimantis aff. calcaratus (N = 2)		-	-	-	0.0004 ± 0.00	-	-	
H. rugulosus (N = 3)	Muscle	-	25.20 ± 19.42	44.35 ± 25.63	-	6.13 ± 4.57	8.14 ± 7.93	Intamat et al. (2016)
F. limnocharis (N = 4)		-	20.41 ± 5.61	82.10 ± 35.17	-	3.17 ± 2.63	3.17 ± 1.62	

K. pulchra (N = 3)		-	29.20 ± 4.30	323.81 ± 33.21	-	9.63 ± 14.30	2.58 ± 1.50	
M. heymonsi (N = 4)		-	47.73 ± 16.11	78.32 ± 29.30	-	8.33 ± 1.76	2.00 ± 1.05	
M. pulchra (N = 3)		-	48.64 ± 9.04	90.77 ± 32.98	-	4.28 ± 2.90	4.36 ± 3.00	
Boana faber (N = 40)	Liver	164.7 ± 316.01	506 ± 63.25	4747 ± 215.06	-	19.7 ± 30.36	87.4 ± 40.48	Zocche et al. (2013)
	Muscle	174.5 ± 342.31	3.7 ± 5.69	61.9 ± 16.45	-	4.8 ± 5.69	52.1 ± 24.04	

Table 4. Data comparison of concentrations (mg/L) of trace metal(loid)s in water samples across different mining contexts and research areas. Unless otherwise indicated, water samples were filtered using 0.45 μm membrane filters.

Sampling site/Reference	**Al (mg/L)**	**As (mg/L)**	**Ba (mg/L)**	**Cu (mg/L)**	**Fe (mg/L)**	**Mn (mg/L)**	**Sr (mg/L)**	**Zn (mg/L)**	**Country**	**Notes**
J1 - This study	0.168	<0.01	0.063	0.22	<0.01	0.037	0.036	<0.01	Ecuador	
J2 - This study	0.139	<0.01	0.032	0.207	<0.01	0.025	0.056	<0.01		
J3 - This study	0.053	<0.01	0.037	0.135	<0.01	0.024	0.106	<0.01		
GM - This study	0.164	<0.01	0.033	0.144	0.071	0.032	0.082	0.399		

J4 - This study	0.05	<0.01	0.035	0.159	<0.01	<0.01	0.015	<0.01		
J5 - This study	0.035	0.029	0.028	0.167	<0.01	<0.01	0.013	<0.01		
J9 - This study	0.064	<0.01	0.03	0.177	<0.01	<0.01	0.013	<0.01		
FA - This study	0.112	0.039	0.046	0.004	0.011	<0.01	1.084	<0.01		
FB - This study	0.878	<0.01	0.036	0.341	0.038	0.039	0.068	<0.01		
Adlassnig et al. (2013)	-	-	-	0.0016 ± 0.001	-	0.24 ± 0.31	-	0.029 ± 0.001	Romania	Acidic drainage from mine stulm related to gold mining. Also silver, copper, and mercury were extracted. No information about samples being filtered
Intamat et al. (2016)	-	0.22 ± 0.01	-	0.021 ± 0.008	2.62 ± 0.68	1.33 ± 0.06	-	0.12 ± 0.08	Thailand	Gold mining, samples filtered through a 11 µm membrane filter

Marques et al. (2011)	3.23	0.002	0.011	0.021	2.746	4.772	0.227	0.357	Portugal	Uranium mine, no information about samples being filtered
Mol & Ouboter (2004)	0.20 ± 0.17	-	-	-	-	-	-	-	Suriname	Wet season. Gold mining
Tarras-Wahlberg et al. (2001)	-	-	-	0.088	-	-	-	0.02	Ecuador	Small scale gold mining. PU-1 location in wet season 1998
	-	-	-	0.0007	-	-	-	0.0015		Small scale gold mining. PU-2 location in wet season 1998
	-	-	-	1.5	-	-	-	0.021		Small scale gold mining. PU-1 location in dry season 1998

	-	-	-	0.023	-	-	-	0.034		Small scale gold mining. PU-2 location in dry season 1998
	-	-	-	0.029	-	-	-	0.03		Small scale gold mining. PU-1 location in wet season 1999
	-	-	-	0.0038	-	-	-	0.029		Small scale gold mining. PU-2 location in wet season 1999
	-	-	-	3.3	-	-	-	0.37		Small scale gold mining. PU-1 location in dry season 1999
	-	-	-	0.037	-	-	-	0.083		Small scale gold mining. PU-2 location

										in dry season 1999
Wood & Williams (2013)	0.101	-	-	-	0.197	0.0399	-	-	United States of America	Mountaintop surface mines

Table 5. Data comparison of various water parameters across different mining contexts and research.

Sampling site / Reference	**EC (µS/cm)**	**DO (mg/L)**	**pH**	**T (°C)**	**TDS (mg/L)**	**Turbidity (NTU)**	**Alkalinity (mg $CaCO_3$/L)**	**Total hardness (mg $CaCO_3$/L)**	**Chloride (mg Cl/L)**	**Sulfate (mg SO_4/L)**	**Notes**
J1 - This study	34.23	10.24	5.99	16.7	26.43	0.87	9.6	3.64	1.15	10.5	
J2 - This study	41.13	10.01	5.99	16.67	31.85	0.46	10.1	3.33	1.1	16.5	
J3 - This study	63.37	9.5	6.07	17.1	48.75	0.885	10.1	2.8	1.15	25	
GM - This study	50.5	9.57	5.96	16.9	39.43	0.425	9.09	3.47	1.25	19.5	
J4 - This study	28.4	10.42	6.69	16.6	22.1	0.135	9.09	3.21	1.15	2	

J5 - This study	29.33	9.62	6.71	16.1	22.75	0.03	10.1	3.23	1.05	2	
J9 - This study	30.02	10.31	6.39	16.02	23.4	0.08	10.1	2.98	1	1.5	
FA - This study	174.9	10.03	6.84	21.77	116.6	0.075	40.4	2.73	1.1	155	
FB - This study	65.47	10.02	5.72	16.9	50.92	0.075	2.02	3.73	1.1	18.5	
Mol & Ouboter (2004)	29 ± 10.3	6.1 ± 1.2	5.6 ± 0.6	25.7 ± 1.3	33.2 ± 16.9	424 ± 377	3.9 ± 4.6	4.7 ± 4.5	5.39 ± 2.04	0.72 ± 0.6	Wet season. Gold mining
	34.7 ± 10.7	1.7 ± 1.2	5.4 ± 0.7	25.4 ± 1.1	22.8 ± 14.9	2874 ± 4885	2.8 ± 2.8	9.3 ± 7.4	6.12 ± 2.36	3.38 ± 0.59	Dry season. Gold mining
Muncy et al. (2014)	1477	-	6.08	13.44	-	-	-	-	-	506.7	Mountaintop removal mining
Pond et al. (2008)	1023 (159-2540)	-	7.9 (6.3-8.9)	11.7 (7.8-18.2)	-	-	-	-	-	-	Coal mining
Simonin et al. (2021)	679 ± 64	11.96 ± 0.07	7.87 ± 0.05	-	-	-	-	-	-	-	Coal mining

Wood & Williams (2013)	1148	-	7.53	-	866	-	-	-	-	-	Mountaintop surface mines

FIGURES

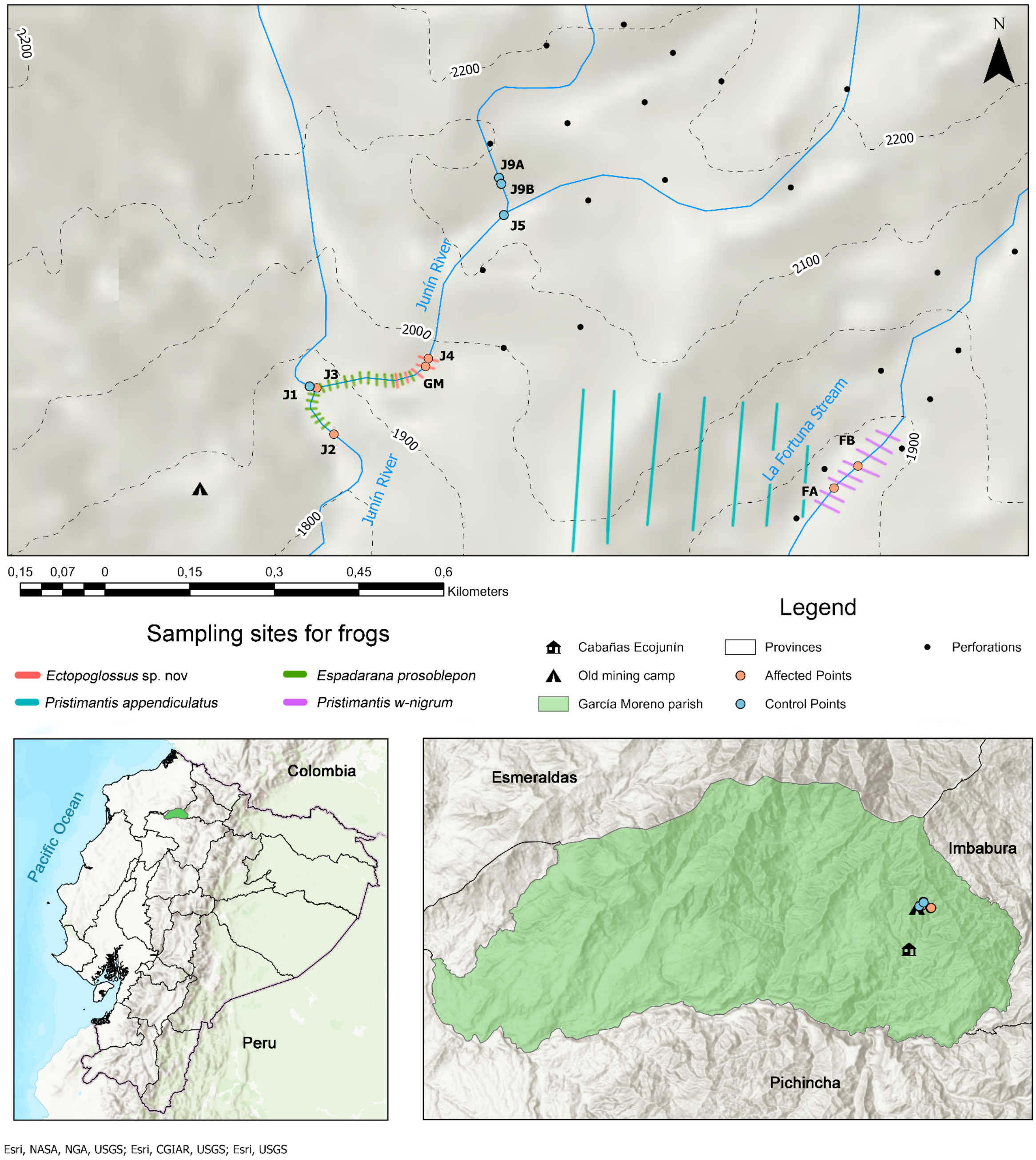

Figure 1. Map of the study area in the Junín Communal Reserve, Intag, Imbabura Province, Ecuador. The top map details the locations of water sampling points along the Junín River and La Fortuna Stream, categorized as control (healthy) and affected (allegedly impacted by mining activities), and the sampling zones for each studied frog

species. Preliminary locations for potential drilling (initial ENAMI exploratory program) are also shown. The old mining camp is shown as a reference point. The left bottom map illustrates the García Moreno parish, highlighted in green in the Imbabura province, in Ecuador. The right bottom map illustrates the location of the sampling points within the García Moreno parish (highlighted in green), with the old mining camp and Cabañas EcoJunín as reference locations.

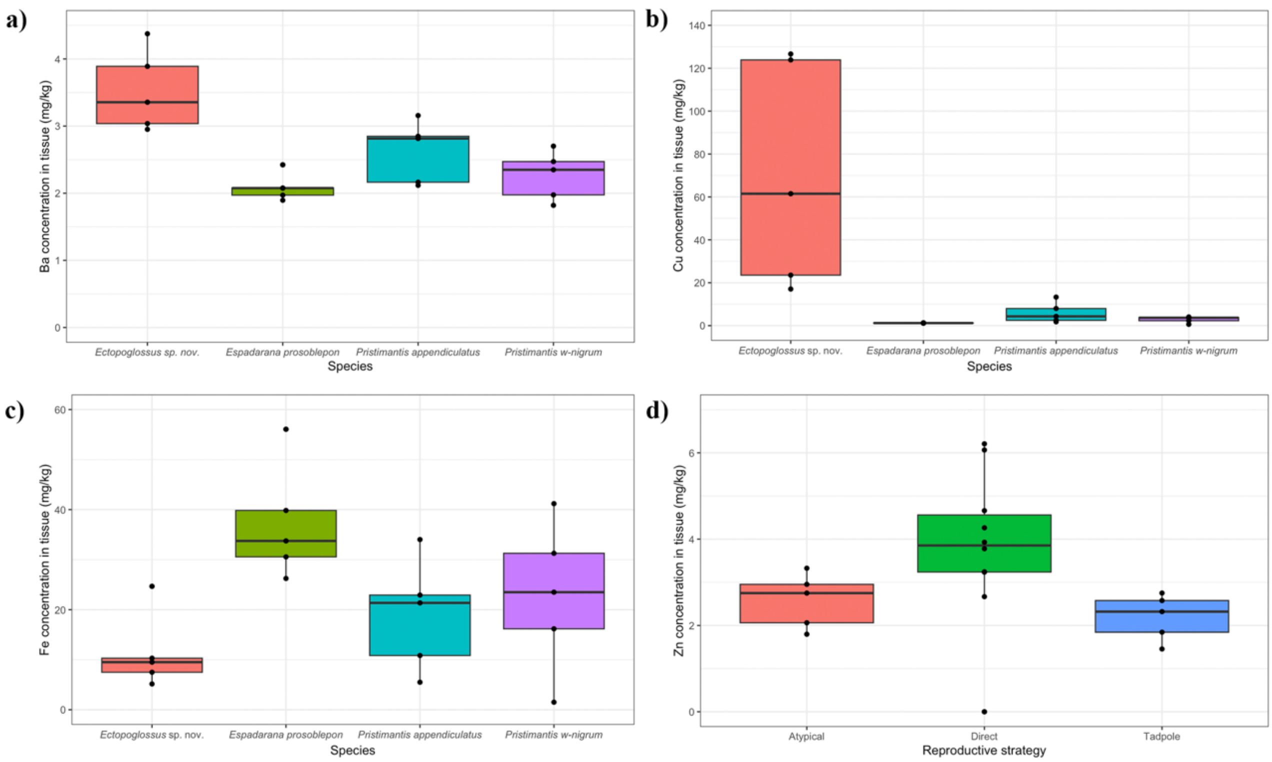

Figure 2. Metal concentrations in frog tissues, values are expressed in milligrams of metal per kilogram of tissue (mg/kg). The box represents the interquartile range, the horizontal line within the box indicates the median, the points represent the data, and the vertical lines extend to the maximum and minimum values excluding outliers. a) Barium (Ba) concentration in the tissues of the four amphibian species studied (N = 5 per species); b) Copper (Cu) concentration in the tissues of the four amphibian species studied (N = 5 per species); c) Iron (Fe) concentration in the tissues of the four

amphibian species studied (N = 5 per species); d) Zinc (Zn) concentration in the tissues of the three reproductive strategies (N = 5 for atypical and tadpole strategies, and N = 10 for direct development).

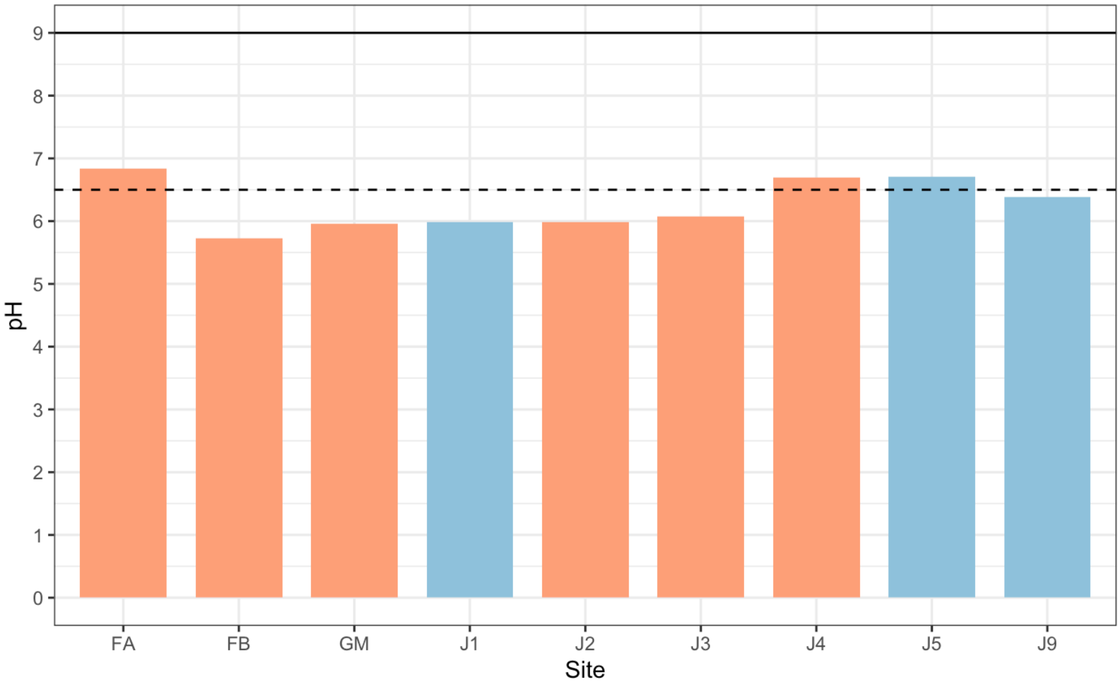

Figure 3. Bar plot of the pH measured at each sampling point (N = 9). Bars are colored with blue for control sites and orange for affected locations. The black dotted line indicates the minimum value (pH = 6.5) allowed by the Ecuadorian legislation, while the black solid one indicates the maximum value (pH = 9) allowed by the Ecuadorian legislation (Table 2 from the TULSMA).

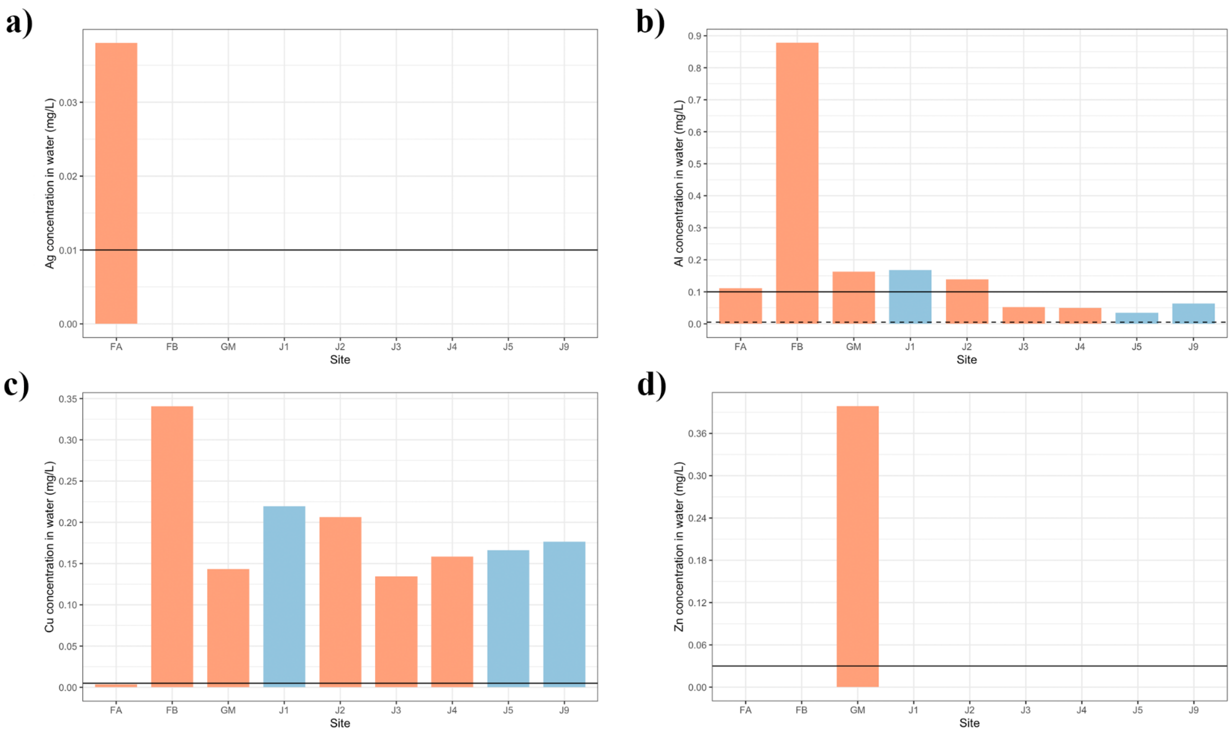

Figure 4. Bar plot of metal concentrations measured at each sampling point (N = 9). Values are expressed in milligrams of metal per liter (mg/L). Bars are colored with blue for control sites and orange for affected locations. The horizontal black lines indicate the maximum concentrations allowed by the Ecuadorian legislation (MAE, 2015).

a) Silver (Ag) concentrations measured compared with the maximum concentration allowed (0.01 mg/L).

b) Aluminum (Al) concentrations measured. The solid black line (0.1 mg/L) indicates the maximum concentration allowed when pH is higher than 6.5 (points FA, J4, and J5), while the dotted one indicates the maximum concentration allowed (0.005 mg/L) when pH is lower than 6.5 (points FB, GM, J1, J2, J3, and J9).

c) Copper (Cu) concentrations measured compared with the maximum concentration allowed (0.005 mg/L).

d) Zinc (Zn) concentrations measured compared with the maximum concentration allowed (0.03 mg/L).

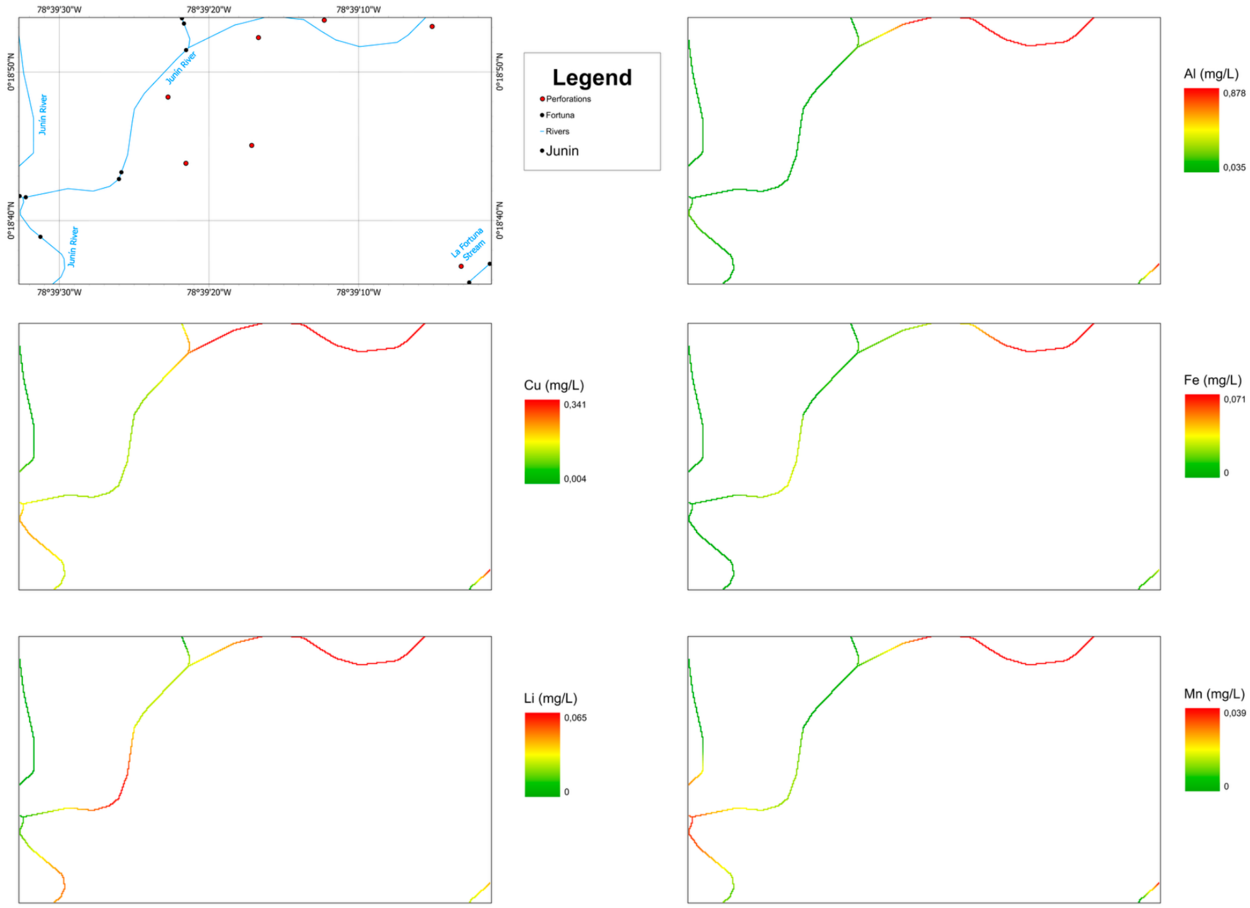

Figure 5. Spatial dispersion models of metals generated using Ordinary Kriging. The figure presents six maps: the top left details the spatial interpolations' area with sampling locations (black points) and preliminary drilling holes (red points) along the Junín River and La Fortuna Stream. The remaining maps illustrate the interpolated concentration values (mg/L) for: Al (top right, range: 0.035-0.878), Cu (middle left, range: 0.004-0.341), Fe (middle right, range: 0-0.071), Li (bottom left, range: 0-0.065), and Mn (bottom right, range: 0-0.039).

SUPPLEMENTARY INFORMATION

Table 1. Values of additional water parameters in all water sampling points. ND = No detected.

Sampling site	Atmospheric pressure (mm Hg)	COD (mg/L)	Nitrite (mg NO2/L)	Nitrate (mg NO3/L)	Phosphate (mg PO4/L)	Fluoride (mg F-/L)
J1	627,20	ND	0,009	2,66	0,48	0,16
J2	627,40	4,01	0,009	3,10	0,37	0,16
J3	627,23	ND	0,007	0,00	0,24	0,16
GM	623,97	ND	0,010	0,00	0,28	0,18
J4	621,80	ND	0,012	0,00	0,20	0,15
J5	615,33	ND	0,010	0,00	0,56	0,09
J9	614,30	3,13	0,02	0,00	0,18	0,04
FA	616,50	ND	0,010	0,00	0,65	0,61
FB	616,00	ND	0,010	0,00	0,07	0,02

Table 2. Values of additional water metal(loid) concentrations in all water sampling points.

Sampling site	Ca (mg/L)	K (mg/L)	Mg (mg/L)	Na (mg/L)	P (mg/L)	S (mg/L)	Li (mg/L)	Si (mg/L)
J1	3,221	0,5305	1,3595	3,7495	0,331	3,9815	<0,01	5,0935
J2	5,664	0,5335	0,4245	1,1135	0,055	5,9125	0,028	4,8545
J3	8,537	0,6225	0,4905	1,9395	0,071	9,8065	0,018	4,8425
GM	7,174	0,6155	0,4295	2,2335	0,236	7,5535	0,065	4,8255
J4	1,166	0,5995	0,1565	0,2905	0,072	0,7375	0,036	4,7515
J5	0,876	0,3465	0,1295	0,2425	0,183	0,5815	0,026	4,4515
J9	1,07	0,569	0,1495	0,196	0,0555	0,328	0,0015	4,5555
FA	62,467	0,9345	0,3435	15,4635	0,323	65,0565	0,027	9,9205
FB	3,391	0,5515	0,2425	2,2015	0,056	8,4245	0,039	5,7815

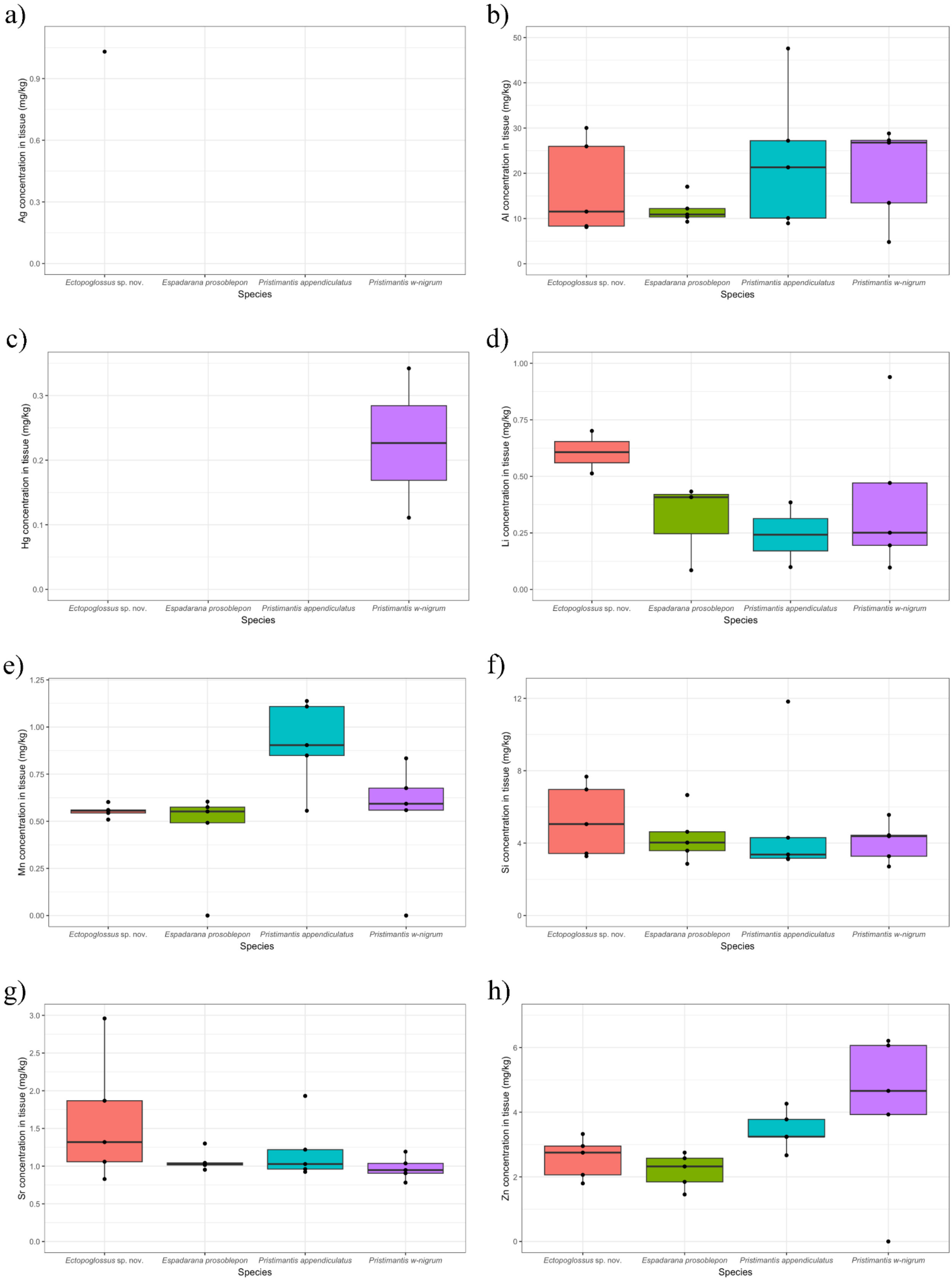

Figure 1. Metal concentrations in frog tissues, values are expressed in milligrams of metal per kilogram of tissue (mg/kg). The box represents the interquartile range, the

horizontal line within the box indicates the median, the points represent the data, and the vertical lines extend to the maximum and minimum values excluding outliers. a) Silver (Ag) concentration in the tissues of the four amphibian species studied (N = 5 per species); b) Aluminum (Al) concentration in the tissues of the four amphibian species studied (N = 5 per species); c) Mercury (Hg) concentration in the tissues of the four amphibian species studied (N = 5 per species); d) Lithium (Li) concentration in the tissues of the four amphibian species studied (N = 5 per species); e) Manganese (Mn) concentration in the tissues of the four amphibian species studied (N = 5 per species); f) Silicon (Si) concentration in the tissues of the four amphibian species studied (N = 5 per species); g) Strontium (Sr) concentration in the tissues of the four amphibian species studied (N = 5 per species); h) Zinc (Zn) concentration in the tissues of the four amphibian species studied (N = 5 per species).