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An integrative approach to identify the impacts of multiple metal contamination sources on the Eastern Andean foothills of the Ecuadorian Amazonia

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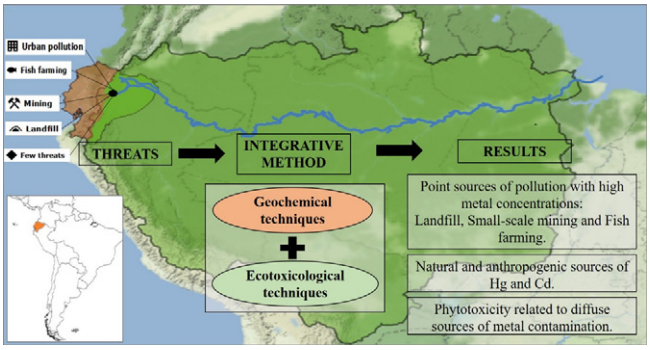
HIGHLIGHTS

- Point pollution sources are significant sources of metal, including landfills, small-scale mining and fish farming.
- Hg and Cd originated from both natural and anthropogenic sources.
- Phytotoxicity was related to diffuse sources (urban pollution and landfill).

GRAPHICAL ABSTRACT



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ABSTRACT

Currently, several concerns have been raised over metal contamination in the upper Amazon basin. Rivers that flow from the high Andes to the lowland Amazon are threatened by anthropogenic activities, which may, in turn, lead to increased metal concentrations in both water and sediments. In the present study, the impacts of multiple metal contamination sources in these ecosystems were identified. The degree of metal contamination was assessed in water and sediment and seed phytotoxicity analyses were carried out in samples taken from 14 sites located in upper Napo River tributaries, combining geochemical and ecotoxicological techniques. These tributaries were chosen based on their degree of anthropogenic contamination and proximity to known sources of relevant pollution, such as small-scale gold mining (MI), urban pollution (UP), fish farming (FF) and non-functional municipal landfill areas (LF). Our results suggest that anthropogenic activities are introducing metals to the aquatic ecosystem, as some metals were up to 500 times above the maximum permissible limits for the preservation of aquatic life established by Ecuadorian and North American guidelines. Sites located close to small-scale gold mining and sanitary landfills presented 100 to 1000 times higher concentrations than sites classified as "few threats". In water, Cd, Pb, Cu, Zn and Hg were mostly above the maximum permissible

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Urban pollution
Mining
Fish farming

limits in the samples, while Cd in sediment reached concentrations 5-fold above the probable effect level (PEL). Phytotoxicity was associated through the diffuse contamination present in urban and landfill areas. Overall, metal concentrations and phytotoxicity assessments suggest anthropogenic effects to environmental contamination, even though natural sources cannot be disregarded. Anthropogenic effects in the eastern Andean Rivers need to be constantly monitored in order to build a complete picture on how pollution sources may affect this strategic Amazon basin area.

1. Introduction

Currently, several concerns have been raised over the environmental issues that impact the Amazonian biodiversity and landscape (Davidson et al., 2012). Environmental contamination in Amazonia has increased, as pollution sources have multiplied due to increasing population size, economic activity diversification (Pimm et al., 2014) and political decisions that have caused the loss of environmental protection measures (Abessa et al., 2019; Bockmann et al., 2018). The Ecuadorian Amazonia comprises about 500,000 inhabitants (INEC, 2010) , where a significant percentage lives in poverty conditions in small towns and villages that lack basic public infrastructure, such as effective dumping areas and sewage treatment, consequently increasing environmental contamination risks (Chiriboga and Wallis, 2010; Vélez, 2015). In addition, some of these activities, such as non-native fish farming, intensive toxic agrochemical use and mining have also contributed in impacts to this region (Pinto et al., 2011; Panday et al., 2015; Roy et al., 2018; Lessmann et al., 2016). All these activities contribute to the increase of metal concentrations in aquatic ecosystems. Most studies have evaluated the impacts of these activities in the Sucumbios and Orellana provinces (Barraza et al., 2018; Pérez et al., 2015; Moreno, 2017), while assessments concerning metal contamination at the Napo province, located in the transition zone between Andes and Amazonia, are still incipient.

Historically, Ecuadorian Amazonia has been impacted by metal contamination through mining and oil extraction subproducts (Pérez et al., 2015; Lessmann et al., 2016). In the Northern provinces, oil extraction began in the mid-1960s and expanded toward the South in the subsequent decades (Valdivia, 2005; Latorre et al., 2015). In Southeastern Amazonia, the presence of both legal and illegal mining (large and small scale) began about 50 years ago (Miserendino et al., 2013; Velásquez-López et al., 2010; Zarroca et al., 2015) and has been one of the main sources of income for the area (López-Blanco et al., 2015), albeit also an important source of contamination. Chemically available metals, such as mercury (Hg), copper (Cu), cadmium (Cd) and zinc (Zn) are mainly linked with mining and oil extraction sub-products, which may be detected at abnormal concentrations in contamination assessments (Mora et al., 2019).

Other sources of contamination include agricultural activities and the disposal of municipal solid wastes. In Amazonian provinces, metals can be traced to the use of fertilizers in local agricultural systems (Hurtig et al., 2003) and monocultures (Argüello et al., 2019). Phosphorus-based fertilizers used to improve soil fertility may contain high Cd, Cu, Zn and aluminum (Al) concentrations (Tirado and Allsopp, 2012; Gupta et al., 2014). However, the degree of anthropogenic metal contamination in sediments and waters in the Eastern Andean foothills is still poorly documented. Regarding solid waste disposal, local governments are often unable to provide an effective system for their inhabitants, an issue beyond what a municipal authority can tackle (Sujauddin et al., 2008; World Health Organization, 2015). This aggravates the situation concerning metal concentrations in the environment, since inadequately installed and monitored landfills become relevant point contamination sources. Unfortunately, this is the reality in many Ecuadorian Amazonian urban centers, where disposal areas are commonly located on river margins. Consequently, disposal areas usually accumulate high concentrations of different types of contaminants, and lack of adequate management allows the leachate to contaminate both superficial and ground waters, a problem that can be aggravated in areas presenting a dense river network and high precipitation rates (Encalada et al., 2019).

In order to obtain a broad picture of metal contamination caused by anthropogenic activities, integrative studies involving different lines of evidence, such as ecotoxicology and geochemistry, have been used to assess sediment and water quality in aquatic ecosystems (Araujo et al., 2013; Cruz et al., 2019; Campos et al., 2016). Chemical analyses provide information on the degree and nature of the contamination, whereas ecotoxicological methods detect the occurrence of potential biological effects (Adams et al., 1992; Chapman et al., 2002; Holt, 2000). Among ecotoxicological methods, phytotoxicity bioassays with *Lactuca sativa* L. are widely used, as they are reliable, cost-effective, quick, and simple (Valério et al., 2007), enabling the identification of different levels of phytotoxicity and detecting areas displaying contamination caused either by anthropogenic activities or natural conditions (Torres, 2003).

The eastern Andean foothills are hardly included in regional contamination assessments (e.g. Siqueira et al., 2018). This region presents precipitation rates above 4000 mm/yr, which waters flow through a dense river network that drains from the steep Andean slopes toward Amazonian lowlands. These rivers flow mostly through protected areas when in the mountains, although as soon as they reach the lower elevation areas outside reserves, anthropogenic activities can be found on rivers margins. Most mountain rivers are free-flowing and connected to the main Amazon River without impediments (Grill et al., 2019). Higher metal contamination in the upper parts of the river network may affect the lower parts of the Amazonian river network and should, thus, be constantly monitored. Given the importance of this region, this study aims to evaluate metal contamination in the area and compare detected levels with international and national standards in order to identify potential threats related to anthropogenic activities. An integrative investigation is proposed herein by combining chemical and ecotoxicological analyses.

2. Methodology

2.1. Study area

The study area is located in the Ecuadorian Amazonia, in the eastern Andean foothills. Important local urban centers are Tena, Puerto Napo and Archidona (Fig. 1). The river network in the area is connected to the upper Napo River, which is the main Northern Ecuadorian Amazon River. The aforementioned urban centers are located on the river margins, and have caused direct impacts on rivers banks and on local hydrological and morphological characteristics. The study area comprises about 7.000 ha and a population of ca. 100,000 inhabitants distributed in both rural and urban areas, with a population density of 11.8 inhabitants/km^2 (INEC, 2010).

The sampling stations were located on the Rivers Tena, Colonso and other small tributaries. These rivers are all Misahuallí River tributaries, with the latter a tributary of the Napo River. All rivers have their headwaters in the Andes (Encalada et al., 2019).

Water and sediment samples were collected at 14 stations (Fig. 1, Table 1) in November 2018. Precipitation is above 4000 mm/yr and no months present <100 mm of precipitation. No precipitation above 10 mm was recorded five days prior to the sampling and on the

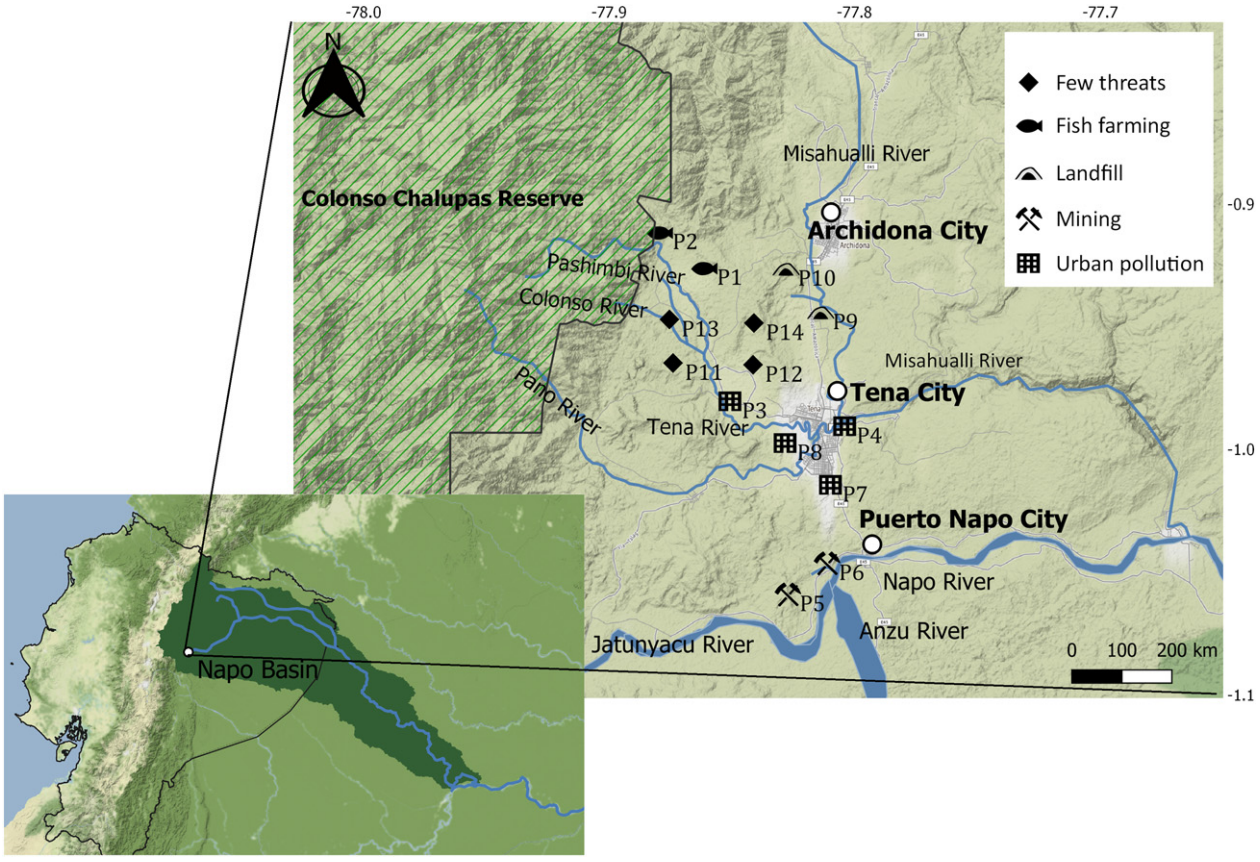

Fig. 1. Geographic location of the 14 sampling stations (coordinates are presented in Table 1) and the locations of anthropogenic activities. Terrain complexity is shown in the background. Only the main rivers that connect to the upper Napo River are highlighted in the figure.

Table 1
Geographic coordinates and physico-chemical parameters of the analyzed water samples from each of the 14 sampling points located on different Napo River tributaries in the Napo province, Ecuador (see Fig. 1 for site locality). Anthropogenic threats are classified as mining (MI), fish farming (FF), waste discharge in urban areas (UP), landfills (LF) and other few threats (FT).

Site	Threat	pH	Conductivity (μS/cm)	Temperature (°C)	Latitude	Longitude
1	FF	8.8	31	31.5	−0.93360	−77.87049
2	FF	7.9	40	28.6	−0.93313	−77.87096
3	UP	7.9	25	28.6	−0.99086	−77.81184
4	UP	7.6	185	27.8	−1.05164	−77.91510
5	MI	7.6	41	28.9	−1.05001	−77.81164
6	MI	7.9	27	28.6	−1.05001	−77.81164
7	UP	7.7	173	27.3	−0.99761	−77.91915
8	UP	7.5	180	27.7	−0.99536	−77.81293
9	LF	8.0	927	29.6	−0.93898	−77.82424
10	LF	7.7	378	31.0	−0.93898	−77.82424
11	FT	8.3	23	24.4	−0.95151	−77.86456
12	FT	8.0	40	23.3	−0.95247	−77.86269
13	FT	8.0	25	24.5	−0.94848	−77.86433
14	FT	7.9	23	24.1	−0.94778	−77.86440

collection day, while during the sampling rainfall reached below 15 mm, which is not enough to characterize flooding in the region (Meteorological Station of Ikiam University, http://meteorologia.ikiam.edu.ec). Altitudinal differences between sampling stations range from ca. 430 m a.s.l. to 660 m a.s.l. and the maximum distance between sites was of about 13 km. To avoid excessive variations in slope, temperature and elevation, only river segments found in lower terrain areas were sampled. Sampling sites were chosen based on their potential anthropogenic contamination degree given their proximity from these pollution sources, including small-scale gold mining (MI), urban areas (UP), fish farming (FF), landfill areas (without waste treatment) (LF), and other few threats (FT). Sampling was always carried out in the streams located immediately after the outfall of the source or in the closest accessible location down from the source (e.g. road bridges). UP sampling was carried out near a waste water outfall on both sides of the river. At FF and LF, sampling was performed on the streams that directly receive the discharge from these sources and which have no other identifiable pollution source upstream. Physico-chemical parameters such as temperature, conductivity and pH were measured in situ employing an HACH HQ11d equipment with a pH and temperature (IntelliCAL™ PHC101) and conductivity probes (CDC40101).

2.2. Water and sediment samples

Single superficial water samples (100 mL) and surface sediments (about 150 g) were collected at the river banks and conditioned in plastic containers. Surface water and bottom (underwater) sediment were collected. Small and shallow rivers were chosen, where the contamination is directly discharged, to avoid the contamination dilution effect. The samples were then transported under refrigeration to the laboratory in a sealed plastic container. At the laboratory, aliquots (about 20 g) for the ecotoxicological bioassays were stored at 4 °C for one day until the experiments were performed. For the metal analyses, aliquots of both sediment and water were stored at 4 °C prior to analyses. Water samples were preserved using HNO_3 (3% *v*/v).

2.3. Total metal(loid) analyses

Total metal(loid) water and sediment analyses were performed after acid digestion according to standard methods (EPA 3050 and 3052) using nitric acid (HNO_3), hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2). The samples were then analyzed using a Thermo Scientific iCAP 7400 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Calibration curves were created from a multi-element standard ICP solution 6VIII, grade Trace CERT Certipur (Merck – Millipore Sigma-Aldrich, US), at concentration 100 mg L^{-1}.

A direct mercury Milestone (DMA 80) analyzer was used for mercury quantification. Based on the EPA 7473 method, a calibration curve was created using a 10 mg L^{-1} mercury standard (Inorganic Ventures). Quality control for major and trace elements analysis was conducted by employing certified reference water (CRM 1640a) and sediment (CRM 1646a) (NIST, Gaithersburg, Maryland) material every 10 samples, at the beginning and at the end of each 16 water sample batch. Recovery percentages were calculated to determine possible matrix effects and to measure method accuracy. All major and trace metal concentrations were corrected based on the recovery percentages obtained in each analysis, ranging from 69% to 93% for sediments and 91–100% for water.

2.4. Phytotoxicity sediment and water sample tests

Lettuce *L. sativa* seed germination and root elongation were tested using water and sediment samples. Germination tests were performed according to USEPA (1996) and OECD (2006) protocols. Fifteen identical seeds were evenly distributed on a filter paper in 90 mm sterile Petri dishes, and 2.5 mL of water samples or distilled water (control) were added. Two replicates were performed for each sample. The Petri dishes were then covered and incubated at 25 °C in the dark for 120 h. For sediment samples, 10 g of sediment were placed in a plastic container (100 mL) and 15 lettuce seeds were evenly distributed on each container. The sediment used as a control was taken from a segment of the Tena River located in a protected area distant from and without any identifiable contamination upstream. The containers were then incubated at 25 °C in the dark for 24 h and maintained under a 12 h/12 h (light/dark) photoperiod for 14 days. At the end of the test, the number of germinated seeds was counted and root lengths were measured.

2.5. Statistical analyses

All results were compared to water quality guidelines established by the Ecuadorian legislation (MAE-TULSMA, 2015), United States Environmental Protection Agency (USEPA, 1996) and Canadian Environmental Quality Guidelines standards were used (CCME, 2002). Only the CCME (2002) criterion was used for sediment samples and concentrations were compared to the threshold effect level (TEL), which represents the concentration below which rare adverse biological effects are expected. The upper value, referred to as the probable effect level (PEL), defines the level above which adverse effects are frequently expected to occur (Long et al., 1995).

Germination and root length percentages compared to the control group were used as phytotoxicity indicators. Prior to the analyses, data were checked for normality and homoscedasticity using the Shapiro-Wilks and Bartlett's tests, followed by comparisons to their respective controls using Student's *t*-tests. The samples were considered toxic when significant differences ($p < 0.05$) between the control and the test samples were detected, leading to, a binary classification toxic or nontoxic sediment and water samples.

Principal Component Analysis (PCA) was used to summarize the main metal concentrations, phytotoxicity and environmental conditions gradients for water and sediments samples. All variables were normalized by site, by setting the sum of squares equal to 1. The first two principal components (PCs) were retained and their correlations to each metal were tested through Pearson's correlation test (Pearson, 1909).

A hierarchical cluster analysis was used to assess the presence of natural groups of sites. This was carried out by an iterative process that defined clusters based on the (dis)similarities of two samples. Dissimilarities between samples were calculated by Euclidean distances and the Group Average Link was used as the agglomeration method in the classification. All statistical analyses were performed using the R software (R core team, 2017).

3. Results

3.1. Physico-chemical parameters

Concerning water physico-chemical parameters, pH values ranged from 6.5–9.0, while conductivity and water temperatures were above the permitted TULSMA and EPA limits (1000 μS/cm, 30 °C) only at site 9 and 10 respectively (Table 1).

3.2. Total metal(loid) content in water and sediment samples

Table 2 and Fig. 2 present the detected metal concentrations in water and sediment samples. Fig. 2 displays the metal values above the Water Quality Criteria for the Protection of Aquatic Life (USEPA, 1993) and Canadian Sediment Quality Guidelines thresholds (CCME, 2002).

3.2.1. Total metal(loid) in water

Cadmium (Cd) content exceeded the permissible limits established by both standards in 71% of the studied locations, with the exception

Table 2
Total metal(loid) contents in water (μg L^{-1}) and sediment (μg g^{-1}) samples collected from 14 sampling stations located at different Napo River tributaries (see Fig. 1 for site locality). Water thresholds: Water Quality Criteria for the Protection of Aquatic Life (USEPA, 1994), showing acute and chronic thresholds, Ecuadorian Guidelines (TULSMA, 2015) and Canadian Environmental Quality Guidelines (CCME, 2002), with short term and long term thresholds. TEL and PEL values originate from Canadian Environmental Quality Guidelines (CCME, 2002).

Sites	Metal concentration in water (μg L^{-1})														CCME		TULSMA	EPA	
	1 FF	2 FF	3 UP	4 UP	5 MI	6 MI	7 UP	8 UP	9 LF	10 LF	11 FT	12 FT	13 FT	14 FT	Short term	Long term		Acute	Chronic
Al	0.3	0.3	0.1	0.2	303.6	7.1	0.3	0.2	4.2	0.4	0.1	0.0	0.1	1.1	–	–	100.0	–	–
Ba	17.4	17.6	26.1	18.3	1883.4	73.2	35.9	40.9	653.4	247.5	23.6	24.5	16.4	30.2	–	–	1000.0	–	–
Ca	1.4	2.1	1.8	2.5	26.9	8.9	23.2	24.8	67.3	98.2	2.9	0.8	2.4	2.6	–	–	–	–	–
Cd	3.9	–	–	1.7	46.0	2.9	–	4.4	10.6	2.5	3.1	2.0	–	2.8	1.00	0.1	1.0	1.8	0.7
Co	–	–	–	–	101.5	–	–	–	6.4	14.6	–	–	–	–	–	–	200.0	–	–
Cr	–	–	–	4.9	238.3	31.5	–	–	9.3	19.4	–	–	–	–	–	–	32.0	–	–
Cu	–	2.8	–	-	135.3	19.2	–	–	–	–	–	–	–	–	2.0	–	5.0	–	–
Fe	0.4	0.3	0.4	0.3	334.1	5.2	0.6	0.6	61.4	4.9	0.2	0.1	0.1	1.1	300.0	–	300.0	–	1000.0
Hg	7.0	11.2	3.9	0.5	–	-	6.7	–	–	–	–	–	–	–	0.026	–	0.2	1.4	0.8
K	2.3	1.7	0.8	0.7	8.8	1.4	1.9	2.1	33.7	318.5	1.2	0.3	0.7	0.9	–	–	–	–	–
Mg	0.5	0.7	0.5	0.5	32.5	2.0	1.3	1.3	6.2	25.0	0.6	0.3	0.6	0.8	–	–	–	–	–
Mn	10.5	31.3	35.9	17.5	2828.4	140.3	61.5	77.6	1466.9	989.3	18.5	5.4	7.7	47.0	–	–	100.0	–	–
Na	2.6	2.9	2.0	1.9	2.6	2.3	4.4	5.1	16.7	150.9	3.7	1.5	3.7	4.1	–	–	–	–	–
Ni	–	–	–	–	155.6	–	–	–	–	18.1	–	–	–	–	–	25.0	25.0	470.0	52.0
Pb	118.1	–	–	5.0	133.1	3.0	–	63.0	49.7	0.7	45.9	1.1	–	32.7	–	–	1.0	82.0	3.2
Zn	2.6	26.4	9.5	2.4	712.0	78.1	5.7	15.2	45.8	25.2	31.2	3.2	10.9	3.2	37.0	7.0	–	120.0	120.0

Sites	Metal concentration in sediment (μg g^{-1})														CCME		TULSMA	EPA	
	1 FF	2 FF	3 UP	4 UP	5 MI	6 MI	7 UP	8 UP	9 LF	10 LF	11 FT	12 FT	13 FT	14 FT	TEL	PEL		Acute	Chronic
Al	121,985.2	269,301.0	11,312.1	82,753.8	6773.7	33,252.4	10,055.9	13,419.1	19,536.5	2880.8	5770.7	27,506.2	6743.2	8776.6	–	–	–	–	–
Ba	140.7	184.3	77.9	640.8	44.9	178.7	93.4	130.5	1574.2	43.2	87.0	115.1	76.2	98.1	–	–	–	–	–
Ca	2709.8	4689.9	4992.6	14,911.8	906.7	3849.8	7572.6	8065.0	42,385.6	5971.3	1124.3	1062.4	1001.9	1638.5	–	–	–	–	–
Cd	2.5	8.0	2.1	4.6	1.6	4.7	2.6	3.1	20.1	1.9	2.8	2.4	1.4	3.1	0.6	3.5	–	–	–
Co	7.8	15.9	5.2	18.6	4.0	11.8	4.7	6.5	8.5	1.4	3.6	6.1	2.9	4.4	–	–	–	–	–
Cu	30.8	40.2	15.9	50.8	4.4	21.9	18.0	20.3	12.8	2.4	2.5	8.4	5.6	5.6	35.7	197	–	–	–
Cr	16.9	38.8	7.5	11.2	8.0	21.3	9.1	12.9	20.6	2.7	4.7	8.7	2.8	7.1	37.3	90	–	–	–
Fe	35,713.5	106,703.8	17,104.2	40,207.3	13,569.5	39,700.7	21,247.3	22,861.5	173,779.8	14,249.8	22,136.1	19,918.8	9309.6	26,349.6	–	–	–	–	–
Hg	0.1	0.4	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	-	0.0	0.0	0.0	0.17	0.486	–	–	–
K	803.4	842.3	940.5	3080.1	201.4	865.2	966.8	764.8	3138.4	857.5	1619.8	430.6	1092.8	1289.4	–	–	–	–	–
Mg	5851.8	7288.5	3062.5	5476.4	996.0	3589.5	3278.7	3782.2	5038.3	944.0	1896.8	3661.8	1690.6	2146.1	–	–	–	–	–
Mn	149.0	468.4	262.7	853.8	229.1	732.4	324.7	338.7	1613.7	279.3	378.4	231.1	301.1	438.0	–	–	–	–	–
Na	278.2	322.0	73.2	1232.7	40.8	54.2	170.0	140.2	366.9	73.4	–	383.4	–	–	–	–	–	–	–
Ni	7.8	11.3	3.5	9.9	4.2	17.2	5.6	7.7	5.4	–	–	3.7	0.9	–	–	–	–	–	–
P	3196.3	4143.4	982.9	8584.9	147.1	924.7	1541.2	2910.7	87,110.2	358.2	289.8	1066.0	399.8	596.5	–	–	–	–	–
Pb	4.2	11.0	1.5	8.9	1.6	5.5	3.7	5.9	6.3	2.6	1.4	1.0	1.3	2.0	35	91.3	–	–	–
Sr	15.3	40.6	19.1	369.0	6.4	23.8	31.1	38.1	344.8	15.9	12.9	12.6	8.7	15.4	–	–	–	–	–
V	183.6	370.7	44.4	170.4	29.6	102.5	38.9	46.2	63.0	10.4	41.7	45.6	17.2	42.8	–	–	–	–	–
Zn	52.3	77.4	39.9	90.2	21.3	60.8	69.7	233.7	81.5	17.8	26.4	44.3	21.0	30.3	123	315	–	–	–

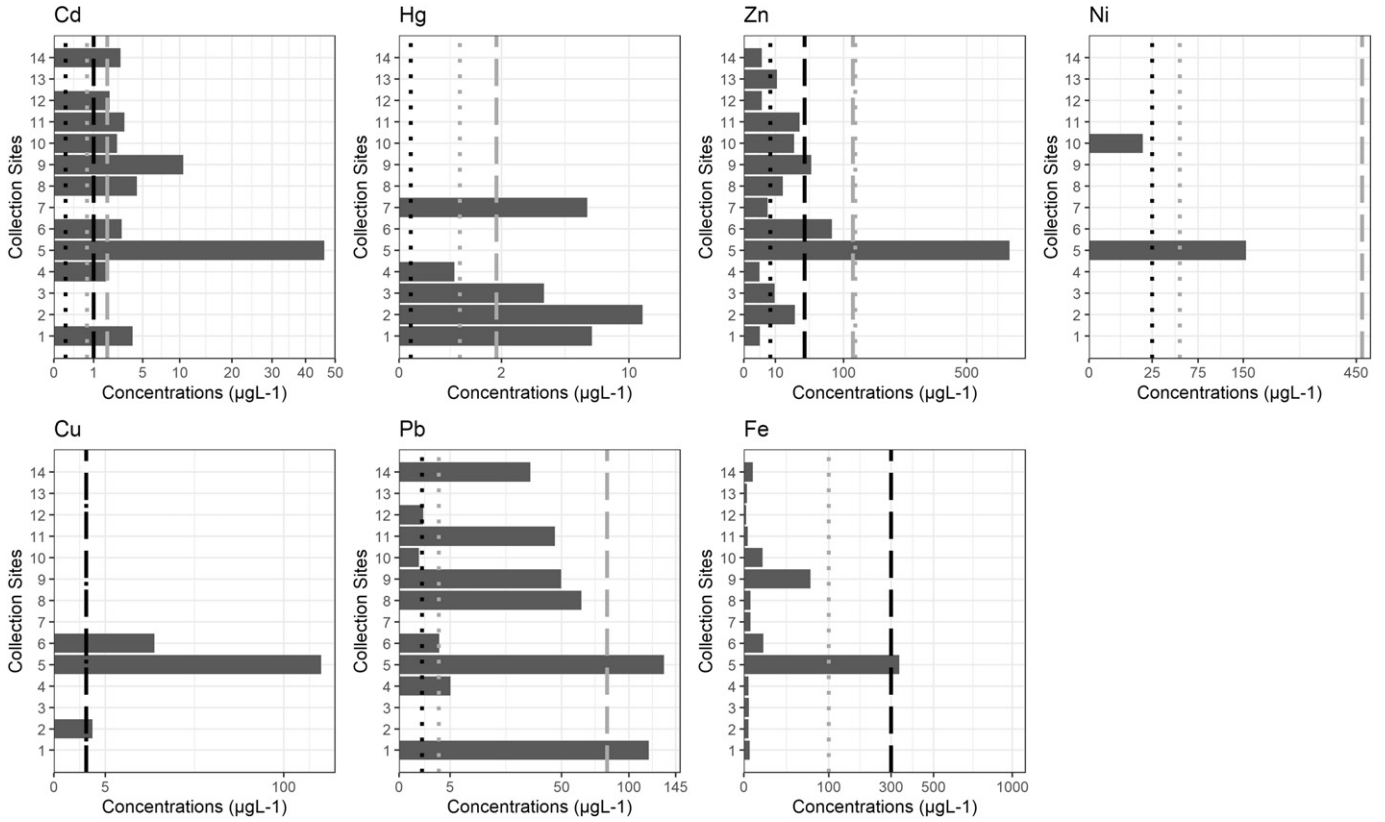

Fig. 2. Metal concentration (µg L^{-1}) in water samples from 14 stations located at different Napo River tributaries. Gray lines indicate the Water Quality Criteria for the Protection of Aquatic Life (USEPA, 1994) thresholds; dashed lines indicate acute limits and dotted lines chronic limits. Black lines indicate Canadian Environmental Quality Guidelines (CCME, 2002); dashed lines indicate short term limits and dotted lines long term limits. All values were square-root-transformed to enable better visualization. Only metal with concentrations above established permissible thresholds levels are shown.

of sites 2, 3, 4 and 13. Samples from sites 5 (MI) and 9 (LF) presented the highest Cd concentrations (46.0 and 10.6 µg L^{-1}, respectively).

Concerning copper (Cu), water from site 5 showed the highest value (135.3 µg L^{-1}), followed by site 6 (MI) (19.2 µg L^{-1}), above CCME and TULSMA thresholds. Site 2 (FF), presented values (2.8 µg L^{-1} Cu) exceeding only the CCME standard.

Lead (Pb) concentrations were higher than the permissible values for USEPA or TULSMA, at 93% of the assessed sites. Higher concentrations were detected in site 1 (FF), followed by sites 5 and 6 (MI) and sites 8 and 9 (LF). Pb concentrations were under the limit of detection or under permissible values in 28.5% of sites (Table 2).

Mercury (Hg) was detected in 35% of the water samples at concentrations above the acceptable thresholds, including at sites 1 and 2 (FF), followed by sites situated in urban areas (3, 4 and 7).

Only the water sample obtained from site 5 (MI) presented aluminum (Al), iron (Fe) and nickel (Ni) concentrations above the permissible limits (Table 2). Zinc (Zn), on the other hand, was detected above the USEPA threshold in one sample (5 MI).

3.2.2. Total metal(loid)s in sediments

A total of 100% of the evaluated sites contained Cd concentrations above the TEL (0.6 µg g^{-1}) (CCME, 2002). Furthermore, sediments from sites 2 (FF), 4 (UB), 6 (MI) and 9 (LF) also exhibited concentrations above the PEL (3.5 µg L^{-1} Cd). Sediment from site 9 (LF) was 5.9-fold above the established PEL (20.1 µg g^{-1} Cd).

Regarding Cu, only sediments from sites 2 (FF) and 4 (UP) presented concentrations above the TEL (35.7 µg g^{-1} Cu). In the case of Cr and Hg, only site 2 (FF) presented values above the TEL (37 µg g^{-1} and 0.17 µg g^{-1}, Cr and Hg respectively). Site 8 (UP) presented values 1.9-fold higher than the TEL (123 µg g^{-1}) for Zn (Fig. 3).

3.3. Phytotoxicity tests

Regarding water phytotoxicity (Fig. 4), individuals exposed to samples from sites 3 (UP), 7 (UP), 10 (LF), 13 (FT) and 14 (FT) displayed inhibited epicotyl growth, indicating sample toxicity. On the other hand, seeds exposed to water from sites 4 (UP) and 11 (FT) displayed enhanced root growth, which may indicate excess organic matter (eutrophication) at these locations.

Regarding sediment phytotoxicity (Fig. 4), epicotyl growth was significantly different than that observed for the control for sites 3, 4, 7, 10, 11, 13 and 14, although the responses were different at each site. Sediments from sites 3 and 4 (UP), 11 and 13 (FT) and 14 (FT) caused increased root growth, while sediments from sites 7 (UP) and 10 (LF) induced epicotyl inhibition, indicating phytotoxicity.

3.4. Hierarchical cluster analysis and PCA

For water samples, the hierarchical cluster analysis indicates that the site 5 (MI) forms a groups separated from all the other sites (Fig. 5). This is probably due to high Cd, Al, Pb, Fe, Zn and Ni concentrations (Fig. 2). Sites 9 (LF) and 10 (LF) formed independent groups. Site 9 presented high Cd, Pb, Fe and Zn concentrations, while site 10 displayed high Cd, Zn and Ni levels. In both cases, all metals were above the threshold limits allowed by the applied US and EC standards.

3.5. Cluster analysis and PCA

For sediment samples, the first cluster analysis division separated site 9 (LF) from the other sites. Site 9 (LF) presented the highest Cd concentrations among all sites. The second division separated site 2 (FF),

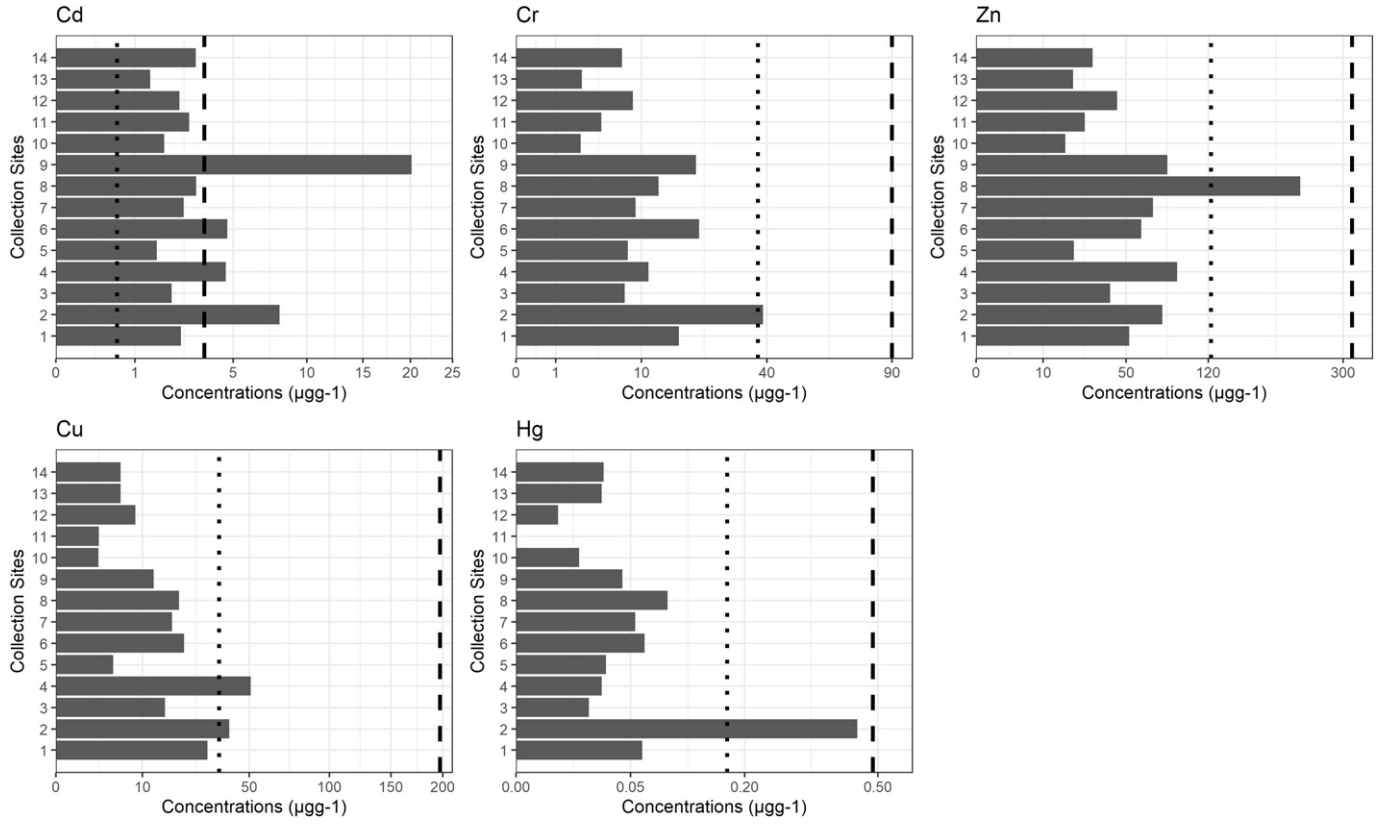

Fig. 3. Metal concentrations (µg g^{-1}) in sediment samples from 14 stations located at different Napo River tributaries. Vertical lines indicate the limits established by the Canadian Environmental Quality Guidelines for the Protection of Aquatic Life (CCME, 2002); dotted lines indicate the threshold effect level (TEL) and dashed lines, the probable effect level (PEL). Concentration values were square-root-transformed to enable better visualization. Only metals with concentrations above established permissible thresholds levels are shown.

presenting the highest Cr, Cu and Hg concentrations and high Cd levels. Sites 9 and 2 presented Cd concentrations above the established PEL. The third division separated site 4 (UP), containing high Cd and Cu concentrations. The remaining cluster grouped all the other assessed sites.

For water samples, the first principal component (PC1) explained 64.26% of the data variance and separated site 5 (MI), which was associated to several trace elements, in accordance to the hierarchical cluster analysis (Fig. 6). The second PC2 explained almost all the remaining variation (20.07%) and separated sites 10 (LF) and 9 (LF), both associated with high levels of several nutrients (Ca, K, Na and Mg). Phytotoxicity was inversely related to nutrients, but was positively associated with some metals, such as Cu, Pb, Hg, Al, Fe and Zn. The third PC apparently joined residual variances. Regarding sediment samples, PC1 explained 49.35% of the data variance. The samples were separated by contamination level in PC1, while PC2 (26.27% variances) separated the samples in two clusters, determined by metal type. Sites 2 (FF), 4 (UP) and 9 (LF), which were the most contaminated, were separated from the rest by PC1, followed by sites 1 (FF), 6 (MI) and 8 (UP). The second PC separated the assessed sites according to type of contaminant and toxicity. Nutrients (Ca, K, P) and some metals (Ba, Cd, Fe, Mn, Sr) and phytotoxicity

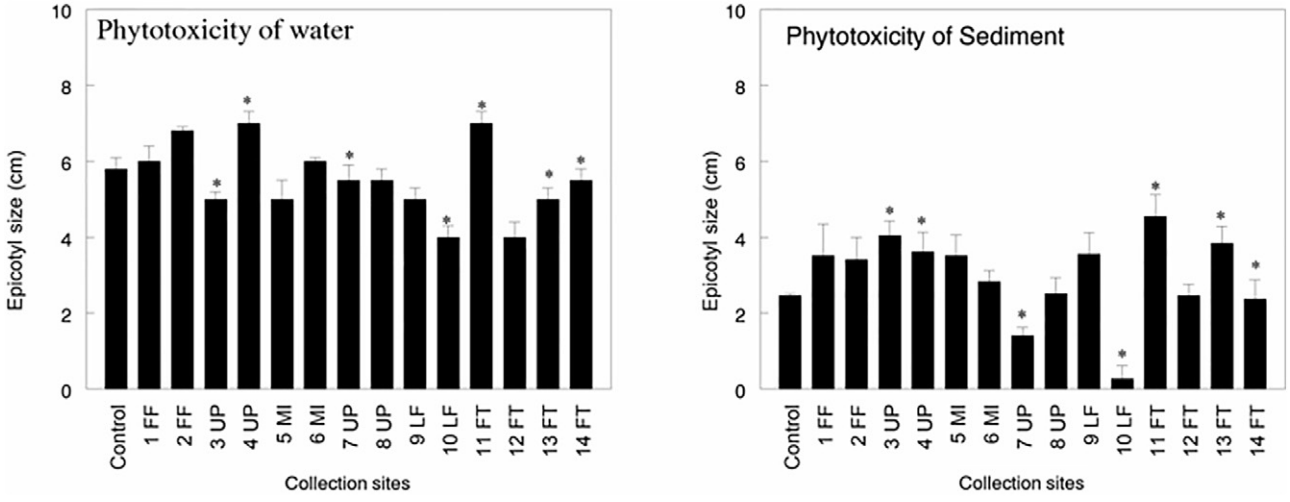

Fig. 4. Results of water and sediment seed phytotoxicity test using Lactuca sativa. Phytotoxicity was evaluated by comparing epicotyl size exposed to water and sediments taken from sampling sites compared to the control using Student's *t*-test. Data are the mean ± standard error ($N = 15$). *indicates significant mean differences ($p < 0.05$).

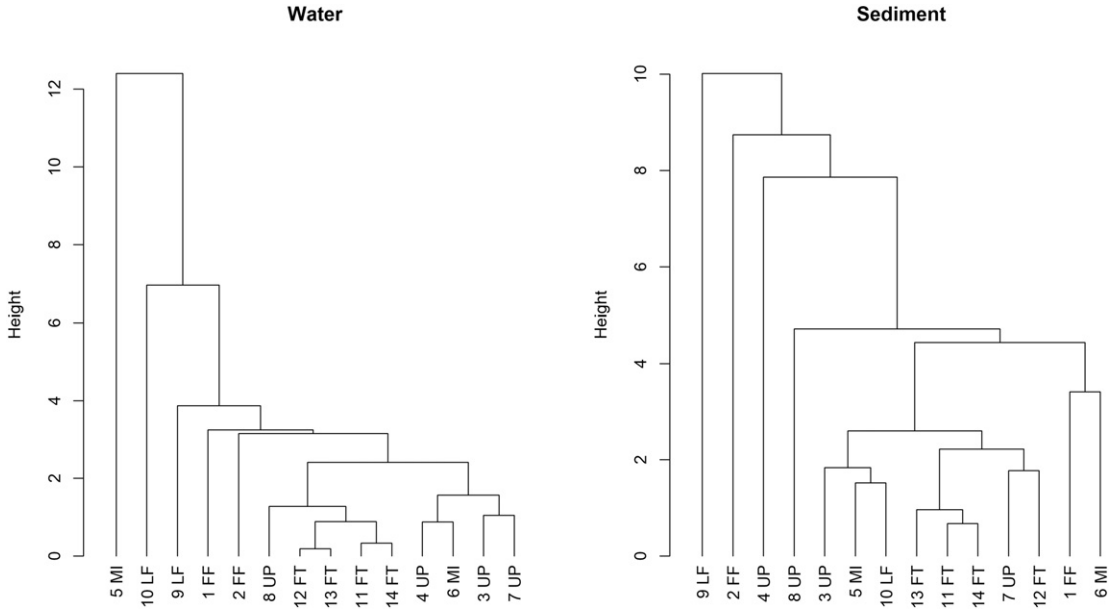

Fig. 5. Hierarchical cluster analysis based on average group links for water (a) and sediment (b) samples. Euclidean distances were used to calculate similarities between the sampling sites. The numbers at the end of the nodes indicate the sampling sites as presented in Table 1.

were negatively correlated in PC2, while most toxic metals (Al, Co, Cu, Cr, Hg, Ni, Pb and V) and Mg presented positive correlations (Table 3). PC3 evidenced phytotoxicity associated to Cu, Co, K, Na, Sr and Zn.

4. Discussion

Metal concentrations above the established thresholds permitted for the protection of aquatic life were found in both water and sediments from fourteen sites located in the Ecuadorian Eastern Andean foothill. The high metal concentrations observed in the present study are probably due to small-scale gold mining, fish farming and non-functional sanitary landfills. In addition, diffuse contamination sources, such as raw sewage, are commonly found in the urban areas and are probably an additional explanatory cause for the detected metal contamination. The integrative assessment carried out in this study, combining metal analyses and phytotoxicity determinations, demonstrates a high contamination degree at all sites located near contamination sources in upper Napo River tributaries.

Gold mining is a frequent activity in the upper Napo River and its tributaries when compared to other Ecuador provinces, such as Zamora or El Oro, or even other Amazon regions (Hacon et al., 2008; Rodrigues-Filho and Maddock, 1997; Moreno-Brush et al., 2016; Roy et al., 2018).

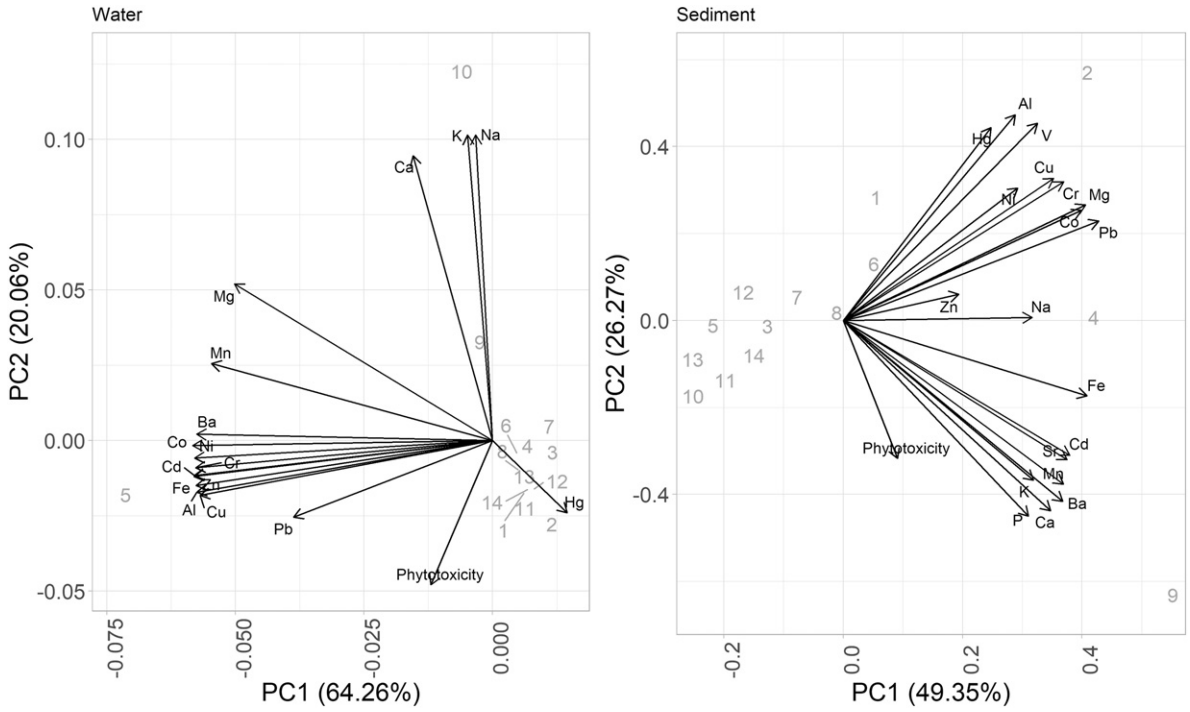

Fig. 6. PCA analysis for the water and sediment samples collected in Ecuadorian Amazon rivers. The dimensional space is determined by the two first PCA axis. The gray numbers represent the collection sites, as indicated in Table 1.

Table 3
Pearson's correlations between the PCA axis and the values of each metal in water and sediment samples.

Water samples			Sediment samples		
	PC1	PC2		PC1	PC2
Phytotoxicity	−0.204	−0.456	Phytotoxicity	0.188	−0.48
Cd	−0.988	−0.111	Al	0.599	0.716
Cu	−0.969	−0.173	Ba	0.763	−0.63
Pb	−0.66	−0.242	Ca	0.721	−0.662
Hg	0.247	−0.228	Cd	0.788	−0.469
Ba	−0.981	0.02	Co	0.829	0.384
Ca	−0.263	0.9	Cu	0.731	0.495
Al	−0.98	−0.163	Cr	0.766	0.484
Co	−0.994	−0.016	Fe	0.847	−0.263
Cr	−0.984	−0.086	Hg	0.514	0.672
Fe	−0.989	−0.114	K	0.662	−0.555
K	−0.083	0.965	Mg	0.843	0.403
Mg	−0.856	0.495	Mn	0.765	−0.57
Mn	−0.931	0.243	Na	0.657	0.01
Na	−0.056	0.965	Ni	0.607	0.461
Ni	−0.986	−0.056	P	0.644	−0.682
Zn	−0.983	−0.142	Pb	0.888	0.347
			Sr	0.779	−0.485
			V	0.675	0.687
			Zn	0.402	0.091

This activity is commonly performed on river banks, and can contribute to increased environmental metal concentrations. Concentrations above permissible thresholds in water were found for Cd, Al, Ni, Fe, Cu, Zn, Ni and Pb in samples from sites 5 and 6 (MI) near a mining concession. Regarding sediment, only Cd was detected above stipulated PEL values. Both sites are no more than 500 m apart, but site 5 (MI) is located on a smaller river (4.5 m width) which receives direct influence of the mining zone and flows toward site 6 (MI), a larger river (11.3 m width). Site 5 presented higher metal contamination than site 6, probably due to differences in river flow and potential metal dilution. A single metal contamination assessment was carried out near these sites by Pérez et al. (2015), where samples taken at the main Napo River channel (width longer than 200 m) were above the permitted limits for K, Fe and Mn. Pérez et al. (2015) sampling, however, cannot be directly affected by mining, as this activity is mainly restricted to small tributaries. The lack of studies in the study region assessed herein is concerning, since new mining concessions have been approved in the area in recent years and tend to increase in the coming years (Roy et al., 2018; Ecuadorian Ministry of Mines, 2017).

In Ecuador, gold extraction originates from both legal and illegal small-scale mining, which takes place in batholith Portovelo-Zaruma (South of the Andes) and Zamora Province (South-east Amazonia) areas (Requelme et al., 2003; Carling et al., 2013; Tarras-Wahlberg et al., 2001). Concentration values for Cd, Cu and Pb detected at sites 5 and 6 were similar to those found in Zamora Rivers, where Mora et al. (2019) detected moderate Cu concentrations and higher Mn, Zn, Pb and Hg values in water samples obtained near mining camps. In the present study, Hg was not detected in the water and concentrations below permissible thresholds were found in sediment samples from sites 5 (MI) and 6 (MI). This is probably explained by the fact that this is a legal mining concession and Ecuadorian Environmental law prevents the use of Hg for gold separation in-situ. Instead, mechanical separation is recommended by Ecuadorian regulatory agencies.

Despite the high metal concentrations observed in the present study, no phytotoxicity was detected in water samples from sites 5 and 6 (MI). This may be due to the chemical speciation of the elements made available by mining activities (Förstner, 2006, Broekaert et al., 2013), leading to non-bioavailability due to less reactive chemical forms (Reeder et al., 2006). Mining activity can potentially induce water and sediment phytotoxicity, due to high toxic trace element concentrations (e.g., Cd, Hg and Cu) and the presence of soluble salts (Tsiridis et al., 2012; Gupta et al., 2014). The degree of toxicity of these metals depends on physico-chemical parameters that induce metal-speciation, such as pH, oxide-reduction potential, hardness and temperature (Reeder et al., 2006; Adriano, 2001; McBride, 1994; Rooney et al., 2006). Metals tend to become more reactive at lower pH, higher oxidation potential, lower hardness and higher temperature (Gambrell et al., 1991). Most of the pH values found in this study were above 7.5 (Table 1). This indicates that some elements may be poorly soluble or present in less reactive forms, associated with hydroxides, oxides and carbonates, silicates, for example. However a more complete diagnosis addressing the different forms of exposure, chronic effects and toxicity to other species are required for the Ecuadorian Amazon.

Sanitary landfills are another important source of metals in the studied region. High Cd, Pb and Zn levels were detected in water and high Cd concentrations were detected in sediments at collection sites 9 (closest to the leach ate outlet point) and 10 (2 m downstream), both located at the sanitary landfill drainage are of the city of Tena. This landfill has already reached its upper capacity and the drainage system has exceeded its maximum limit, producing a leachate that flows directly into a small stream located nearby. The high metal concentrations found in water clearly indicate superficial water contamination due to landfill leachate. For instance, Cd concentrations were above the probable effect level (PEL) in the sediment sample from site 9 (LF), indicating high phytotoxicity and potential bioaccumulations with likely harmful implications for both environmental and human health (Kim et al., 2013; Olafisoye et al., 2013). Site 10 (LF) water and sediment samples presented phytotoxicity, which may be related to metals and a mixture of other compounds, such as organic pollutants like organochlorines and hydrocarbons, and other synthetic substances common in leachate water (Clement et al., 1997; Charles et al., 2011). Na, K and Mg concentrations were also high in water obtained from site 10 (LF), which also exhibited high conductivity (Table 1), suggesting increased water salinity or dissolved ions, which may be associated to phytotoxicity caused by exposure to these metals. Salt toxicity in plants may manifest in several ways. Interference with anion or cation balance can affect membrane integrity and lead to the release of reactive oxygen species (Rout and Shaw, 2001; Simmons, 2012).

Fish farming (FF) sites 1 and 2 presented moderate-to-high Cd, Pb and Hg concentrations in water and Cd, Cr, Hg and Cu in sediments (above the TEL). Some of these metals are common in aquaculture effluents, particularly Cu, Zn, Cd, Mn and Hg (Tacon et al., 2009), originated from feed ingredients, fertilizers, or active principle constituents of chemical compounds applied for different uses (Johnston and Savage, 1991; Boyd and Massaut, 1999; Forster et al., 2003). Sites 1 and 2 (FF) are located in rivers that flow directly from the Colonso-Chalupas Biological Reserve (CCBR) and are used to fill and to receive aquaculture effluent discharges of about 20 fish farming pools (0.2 ha each). Metal concentrations in FF samples were higher when compared to other Amazon areas. For example, the average total Hg in the Amazon Negro River is of 0.0045 μg L^{-1} (Fadini and Jardim, 2001), while the present study detected 7 μg L^{-1} at site 1 (FF) and 11.2 μg L^{-1} at site 2 (FF). Therefore anthropogenic influences cannot be discarded. Concerning sediment, Hg at site 2 (FF) as of 0.4 μg g^{-1} and above the toxic threshold (TEL). Sediments from Amazonian rivers not influenced by anthropogenic activities present Hg concentrations ranging between 0.02 μg g^{-1}- and 0.1 μg g^{-1}, while levels range between 0.5 μg $g^{-1}$and 4.0 μg g^{-1} in contaminated rivers, reaching up to 19 μg g^{-1} (Bastos et al., 2004; Siqueira et al., 2018; Mainville et al., 2006). Thus, it is very likely that Hg is not derived from natural sources at FF sites, but by anthropogenic activities, in agreement with previous assessments carried out in the Brazilian Amazon (Lechler et al., 2000; Siqueira et al., 2018). No phytotoxicity was observed for water and sediment FF samples. Thus, further studies are required to detect the bioavailability of Hg and other contaminants in the area.

Sites 3, 4, 7 and 8 (UP) are located close to urban areas, presenting high Cd, Pb, Hg and Zn levels in water, while sediments exhibited high Cu, Hg, Cd and Zn concentrations. The continuous and direct release of

domestic effluents into the aquatic environment may eventually lead to the accumulation of high metal concentrations in water and sediment, leading to reduced environmental quality (Rocha et al., 2017; Yongming et al., 2006). This is due to the fact that urban wastewater often contains high amounts of organic matter, agrochemicals and organic pollutants (Fent, 1996) and metals such as Cu, Pb, Zn, Ni and Cd (Clement et al., 1997; Martínez and Poleto, 2014). Water and sediment from urban sites 3 and 7 led to phytotoxicity, probably due to discharged sewage or complex mixtures of contaminants present in urban drainage (Marsalek et al., 1999). These results corroborate other studies conducted on the phytotoxicity of urban pollution using *L. sativa* (Bohórquez-Echeverry and Campos-Pinilla, 2007; Hashem et al., 2013), and studies reporting growth inhibition associated with Cu, Pb, Zn, Ni and Cd in acute (artificial) and chronic environmental contamination situations (Azzi et al., 2017; Chan-Keb et al., 2018). On the other hand, contaminants may also stimulate *L. sativa* seed growth by generating stimulatory, (Belz et al., 2018; Tigini et al., 2011; Alvim et al., 2011; Luiz et al., 2013; Di Salvatore et al., 2008), as observed for some water (4 UP and 11 FT) and sediment (3 and 4 UP, 11 and 13 FT) samples assessed herein. The hormesis response phenomenon, characterized by a low dose stimulation and a high dose inhibition (Calabrese et al., 2007) can be observed in all biological responses. At high concentrations, *L. sativa* seed germination inhibition would be expected, while growth stimulation would be expected at low concentrations.

Out of the 14 sampling sites, 10 water samples presented Cd concentrations above threshold levels. The discussion if the main Cd contamination source is natural or anthropogenic in the Amazon is classic and longstanding, as Amazon soils may naturally contain high Cd concentrations, reaching 0.04 μg L^{-1} in water, although international standards indicate adverse effects above 0.01 μg L^{-1} (Ribeiro et al., 2017; Siqueira et al., 2018; Fadini and Jardim, 2001). To disentangle the natural concentrations of this metal from those derived from contamination sources would require the determination of background concentrations, which have not yet been determined for the study area. Therefore, the results reported herein should be compared against the backgrounds of other Amazonian and Andean areas (Barraza et al., 2018; Tarras-Wahlberg et al., 2001; Requelme et al., 2003; Carling et al., 2013; López-Blanco et al., 2015). When compared to commonly used standards (TULSMA, USEPA, CCME), the high values reported herein suggest anthropogenic contamination in the most of the study sites.

Due to lithogenic processes Cd, can be released into the atmosphere through metal production activities, fossil fuel combustion or waste incineration, and can also be enriched in soils by the application of fertilizers or other phytosanitary products (European Food Safety Agency EFSA, 2012; Kirkham, 2006; Pérez et al., 2015; He et al., 2015). In Ecuador, Cd is found in fertilizers and pesticides frequently used in cacao (Barraza et al., 2018) and banana cultivation (Romero-Estévez et al., 2019). These agrochemicals are sold indiscriminately and used without restrictions in the study region. No direct association between high Cd concentrations in the assessed samples and the presence of cultivars can be made, as the sampling stations were not located in rivers directly affected by agricultural activities. However, the phytotoxicity results confirm our hypothesis that Cd can be of anthropogenic origin. Samples from site 14 (FT) inhibited *L. sativa* root growth, while no significant effect was detected for water and sediment samples from site 12 (FT). Site 12 is located on a river that does not flow through agricultural sites, but originates directly from the CCBR, while site 14 is located in a stream that also originates from the CCBR, but flows through agricultural sites.

The complexity of the assessed dense river network metals makes it difficult to map metal sources, even though some are more obvious than others (e.g. 10 LF and 5 MI). The effect of diffuse contamination was detected by phytotoxicity tests, especially in locations close to urban sites, which indicates local biota effects. Point and diffuse metal sources associated with illegal activities, uncontrolled sewage discharges and landfill leachate leakage can contribute to regional contamination, even in low population density areas. Therefore, the high levels of metals found herein and the phytotoxicity of some samples indicate that special attention to environmental metal contamination is required. The sampled sites were chosen in order to evaluate potential first contamination sources right after the assessed rivers flow out of protected areas. Despite such areas displaying low to moderate anthropogenic impacts, metal concentrations in waters and sediments reached moderate to high concentrations. In addition, lack of public sewage treatment policies and of properly installed landfills leads to high metal inputs to aquatic ecosystems and contribute to Amazon River basin contamination.

Overall, the metal concentrations and phytotoxicity reported herein indicate important anthropogenic effects. The multiple point contamination sources present in this study can be identified, including small-scale mining (legal and illegal), sewage discharges, fish farming and non-functional landfills, among others. The phytotoxicity tests were able to detect effects mainly related with urban areas. However, this is the first ecotoxicological approach used in this region and other ecotoxicological and hydrological tools are required to better characterize the environmental quality of the study area, especially to detect possible effects due to the high concentrations of the determined metals. Other important aspects requiring further studies involve metal release from natural origins, due to river bank erosion, effects related to high precipitation rates occurring at the eastern Andean Amazonian foothills, metal contamination impacts of during flooding and drought events and integrative effects on the local biodiversity.

Declaration of competing interest

All authors declare that they have no conflict of interest with any governmental agency or commercial entity.

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