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Assessing the Quality of Amazon Aquatic Ecosystems with Multiple Lines of Evidence: The Case of the Northeast Andean Foothills of Ecuador

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Abstract

We assessed the quality of Andes-Amazonia streams in Ecuador impacted by gold mining (GM), discharges from inefficient sewage network in urban areas (UA), wastes from fish farming (FF) and from non-functional landfill (LF) and other few threats (FT). We selected three lines of evidence (LOE) that were used separately and integrated into a index: water quality (WQI) and macroinvertebrate community (AAMBI) indices and phytotoxicity tests. Streams affected by UA and LF had the lowest scores to WQI and phytotoxicity, and by GM had the lowest scores to AAMBI. Macroinvertebrate absence in GM should be considered as a warning signal of long-term mining impacts in the area. The integrated LOE index showed that sites with identified threats had 30%–53% stream quality decline compared to FT sites. The use of the selected LOE seems to be a useful tools for long-term monitoring and evaluation of this sensitive aquatic ecosystem.

Keywords WQI index · Phytotoxicity · Macroinvertebrate community index · Gold mining · Fish farming · Non-functional landfills · Urban contamination

The Andes–Amazonia ecotone plays a crucial role in regulating the global climate, in the provision of ecosystem services and harbors exceptional biodiversity (Flores et al. 2010; Josse et al. 2009). This area has a dense network of rivers that flow through protected areas, draining from the Andean slopes into the tributaries of the Amazon River (Grill et al. 2019; McClain and Naiman 2008). Although it is an area of global importance, human pressure on this ecosystem has increased in the last decades (Lessmann et al. 2019; Encalada et al. 2019a).

The wastewaters from fish farming, legal and illegal gold mining, urban contamination, and non-functional landfills input high amounts of metals in the freshwater ecosystems at the Northeast Andes foothills of Ecuador (Capparelli et al. 2020). Besides metal contamination, multiple environmental effects can be expected from these contamination sources. For instance, wastewaters from fish farming pools cause eutrophication by diminishing the dissolved oxygen and increasing pH and ammonia (NH_3) (Hussan and Gon 2016); gold mining increases the turbidity, change water color, decrease the pH and affect natural landscapes; inefficient sewage network and the lack of wastewater treatment in urban areas increase organic and microbiological contamination (Flores et al. 2010; Isch 2011; Finer et al. 2008); and the leachate from non-functional landfills contaminates both superficial and groundwater with several types of contaminants. Because anthropogenic activities impact different characteristics of the ecosystem, multiple lines of evidence are required for a complete environmental assessment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00128-020-03089-0.

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The use of multiple lines of evidence (LOE) provides an integrative way to assess environmental risks on the aquatic ecosystems (Burton 2002; Melcher et al. 2016). The choice of the LOE depends on the type of threats, the objectives of the study, and the costs, especially in regions with low scientific investment (Altenburger et al. 2015; Taylor et al. 2010). LOE can combine the assessment of contamination level through chemical analyses and biological responses from key species and/or model organisms at different levels of biological organization (Chapman et al. 2002). Some LOE widely used to assess water ecosystems are the water quality index (WQI), which qualifies the overall water quality within an ecosystem using physical, chemical, and microbiological parameters (Koçer and Sevgili 2014); phytotoxicity tests using *Latuca sativa* L. (U.S. EPA 1996), which assess the toxic potential of water or sediment samples from freshwater ecosystems; and aquatic macroinvertebrates indices, which evaluates water quality based on sensitivity or presence of tolerant taxa (Hering et al. 2006; Stoddard et al. 2008). All these LOE differ systematically in their specificity for chemical pollution, ecological relevance and applicability (Dagnino et al. 2008). However, when integrated, multiple LOE can complement each other and their combination allows the assessment of the degree of impact caused by various contaminants in the freshwater (Santos et al. 2017; Backhaus et al. 2019).

Specific information on the impact caused by multiple contamination sources in the freshwater ecosystem of the Andes–Amazonia ecotone in Northeast Ecuador is scarce. Thus, we aimed at applying three LOE (water and macroinvertebrate quality indexes and phytotoxicity tests) to evaluate water quality of Andes-Amazonia rivers. Each of these LOE has a different approach that, when used together, can draw conclusions on the environmental impacts and be used for future monitoring of Andean-Amazonian freshwater ecosystems.

Materials and Methods

The study area is located in the upper Napo basin, at the Ecuadorian Amazonia (Fig. 1). The Napo River is a tributary of the Amazon River whose headwaters are located in the high elevations of the Andes (Grill et al. 2019). Five sampling sites were located in the Tena and Colonso rivers and in some of their tributaries. The study area comprises a population of approximate 100,000 inhabitants distributed in rural and urban areas (INEC 2010). Annual mean precipitation is above 4000 mm and no months presented less than 100 mm of precipitation. Daily precipitation above 1 mm was not registered six days before sampling. (Meteorological Station of Ikiam University, http://meteorologia.ikiam.edu.ec:3838/meteoviewer/).

Sampling was done in March 2020. Sampling sites were chosen based on the presence of metal contamination sources mapped by Capparelli et al. 2020: sites located close to gold mining and landfills presented 100 to 1000 times higher metal concentrations than sites classified as "few threats". In water samples, Cd, Pb, Cu, Zn and Hg were mostly above the maximum permissible limits in the

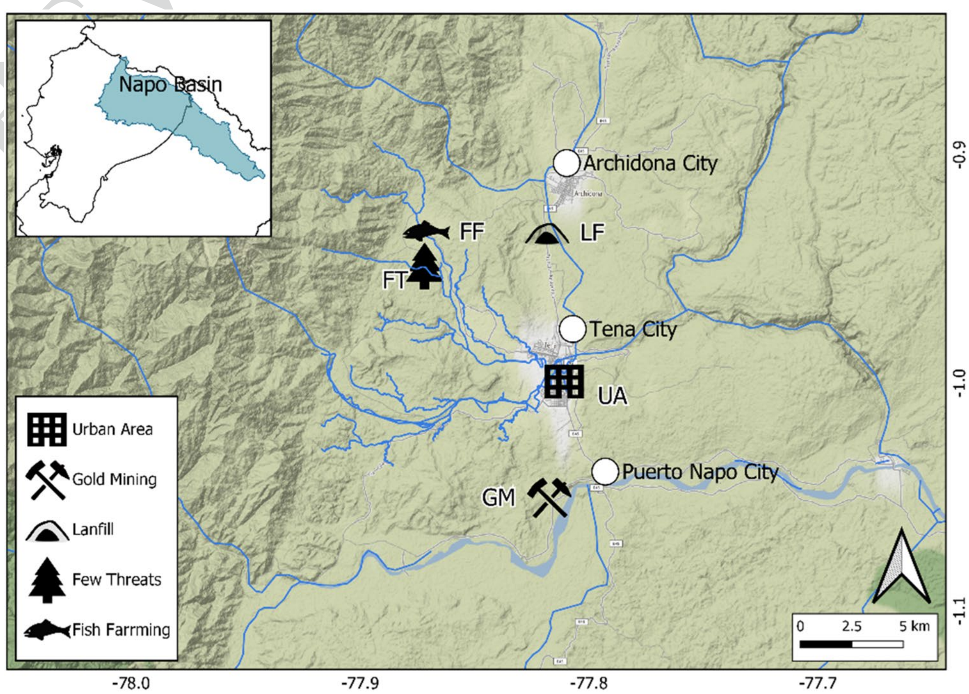

Fig. 1 Geographic location of the sampling rivers as defined by the presence of anthropogenic impacts. Only the main rivers that connect to the upper Napo River are highlighted in the figure. Terrain complexity is shown in the background. The coordinates of the sampling areas are: Few Threats (−77.864, −0.948), Fish Farming (−77.871, −0.933), Gold Mining (−77.817, −1.049), Urban Areas (−77.810, −0.999) and Landfill (−77.818, −0.933)

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samples, while Cd in sediment reached concentrations fivefold above the probable effect level (PEL) in that previous study. Thus, the selected contamination sources were gold mining (GM); urban areas (UA); fish farming (FF); a landfill (LF); and areas where no direct sources of contamination could be identified, named few threats (FT).

LOE 1: The Water Quality index (WQI) is a comprehensive general index for classification of surface water resources based on water quality (Nong et al. 2020; Noori et al. 2018). The index is composed by seventeen parameters: dissolved oxygen, pH, temperature, total dissolved solids, turbidity, chemical oxygen demand, fecal coliforms, color, total phosphates (PO_4^{3}), nitrates (NO_3^-), nitrites (NO_2^-), calcium (Ca^{2+}), magnesium (Mg^{2+}), chlorite (Cl^-), sulphates (SO_4^{2-}), total ammonia (NH_3) and ammonium (NH_4^+), where every parameter have different contribution on water quality. Each parameter was assigned a weight based on its perceived effect on aquatic life to calculate the WQI index (Noori et al. 2018). WQI ranges from 0 to 100. The WQI score is classified in five categories, Excellent (100–91), Good (90–71), Medium (70–51), Bad (50–26), and Very bad (25–0). To determine the parameters used in the WQI, two samples of superficial water were collected in pre-washed low-density polyethylene bottles of 1000 mL, at approximately 30 cm depth from the water surface and against the river flow. The samples were transported in cool boxes and immediately processed upon arrival at the laboratory. The parameters dissolved oxygen, pH, temperature, conductivity, and total dissolved solids were measured in situ using the YSY professional plus multiparameter. Turbidity was read with the HACH TL 2300 turbidimeter. Color apparent was determined by the method 8025 HACH. The chemical oxygen demand was determined by the method 8000. Fecal coliforms were determined using chromocult coliform agar with the most probable number (MPN) technique (APHA 1998), with 3 tubes per water sample dilution (10^{-1} to 10^{-4}).

The total phosphates (PO_4^{3-}), nitrate (NO_3^-), nitrite (NO_2^-), calcium (Ca^{2+}), magnesium (Mg^{2+}), chlorine (Cl^-), sulfate (SO_4^{2}), ammonium (NH_4^+), total ammonia (NH_3), were measured using ion chromatography (Shodex IC-52 4E anion and Shodex IC YS-50 cation). The results were compared to water quality guidelines established by the Ecuadorian environmental guidelines (TULSMA 2015) and the Canadian Environmental Quality Guidelines standards (CCME 2002). Additionally to the parameters used in the calculation of the WQI we also measured in situ electrical conductivity, sodium (Na^+) and potassium (K^+) for the matter of comparison with TULSMA thresholds.

LOE 2: To perform phytotoxicity tests**,** *L. sativa* seed germination and root elongation were tested using both water (150 mL) and superficial sediment (approximately 150 g) from our sampling sites. Samples were collected and stored at 4°C for one month until the experiment was carried out. Seed germination and root elongation were evaluated according to the U.S. EPA (1996) and OECD (2006) protocols. *L. sativa* seeds were purchased from a local seed market (certified sealed seeds with 98% germination rate). Fifteen morphologically identical seeds were evenly distributed on a filter paper in 90 mm sterile Petri dishes, and 2.5 mL of water sample or distilled water (control) were added. Two replicates were performed for each sample. Petri dishes were 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 the FT site. 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 and epicotyl lengths were measured (Hoekstra et al. 2002). Then, germination and root length from both water and sediment experiments were compared to the control group and used as phytotoxicity indicators. Comparisons to their respective controls were done using Student's t-tests. The samples were considered toxic when significant differences ($p < 0.05$) between the test samples and the control were found. Samples were classified as toxic or nontoxic, or with signs of toxicity, when there was greater growth of the root or epicotyl in relation to the control.

LOE 3: To calculate the Andean–Amazon Biotic Index (AAMBI, Encalada et al. 2019b), macroinvertebrates were collected from the benthic substrate at both edges of each stream channel. Samples were taken with a D-shaped net using a multi-habitat approach sampling. River sediment was actively disturbed for one minute at each side of the riverbank and one minute at the center of the river to collect all representative taxa of these sectors. After one-minute sampling the macroinvertebrates were carefully separated in order to not lose the organisms collected in each edge. The samples were placed in plastic bags and transported to the laboratory, where river water was replaced by 96% alcohol. Organisms were identified in all samples and classified to the species level whenever possible (Wright et al. 1984). AAMBI consists in the sum of numerical values assigned to each family; AAMBI values range between 1 and 10, being 1 assigned to the most tolerant families and 10 to the most sensitive families. The final score is then ranked into five categories: excellent water quality (> 121), very good water quality (90–120), good water quality (50–89), regular quality of the water (36–49), and bad water quality with high contamination load (< 35).

By combining the WQI, phytotoxicity and AAMBI, we generated an integrated quantitative index that uses the complete decision matrix for the three LOE with normalized values between 0 and 15 (Table 1).

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Principal Component Analysis (PCA) was used to summarize the main WQI, phytotoxicity and AAMBI gradients. 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.

Results and Discussion

LOE 1: The WQI scores ranged from 43 (LF site) to 89 (FT site) (Fig. 2). None of these sites had the highest possible classification. The UA and LF sites had the worst water quality. Intermediate values were reported for GM and FF sites.

Complete data of physical and chemical parameters can be found in Table 1 of supplementary information. Dissolved oxygen (DO) and chemical oxygen demand (COD) parameters for sites UA and LF (UA = 64.5% DO and 66 mg L^{1}; LF = 16% DO and 99 mg L^{1}) were above the threshold for Ecuadorian guidelines (≥ 80% DO and ≤ 40 mg L^{-1} COD). Low DO can lead to hypoxia and death of aquatic organisms (Cox 2003), while COD most likely increased in these sites due to non-biodegradable organic loading from untreated sewage water and leachate from the landfill. Total dissolved solids (TDS) ranged from 16.5 mg L^{-1} (FT and FF sites) to 146 mg L^{-1} (LF site), being higher than those previously reported to the Napo River basin (64 mg L^{-1} to 99 mg L^{-1}) (Moquet et al. 2016). TDS values were all below the threshold established by CCME guidelines (Table 1 supplementary information). There is a direct correlation between TDS and electrical conductivity (EC, in µS cm^{-1}) (Ustaoğlu et al. 2020). The highest EC value was 293 µS cm^{-1} (LF site) and the lowest was 32.8 (FT site). In the LF site high EC values are likely due to the high load of ions from the leachate water that flow into the nearby stream. In fact, EC values previously reported for the area (Moquet et al. 2016) ranged between 62 µS cm^{-1} and 66 µS cm^{-1}, well below the

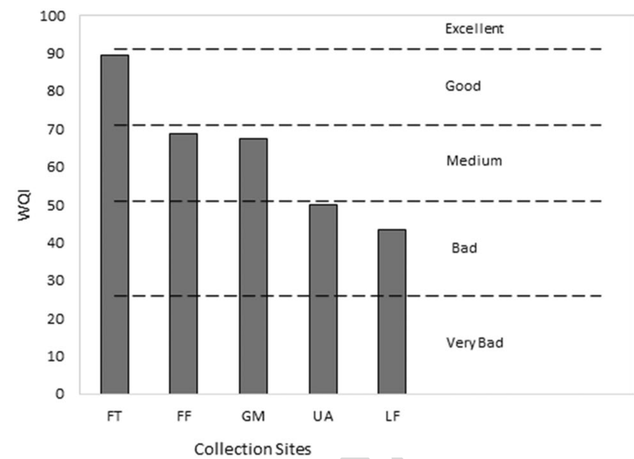

Fig. 2 Water quality index from 5 collection sites located at different Napo River tributaries. Collecting sites are ordered from higher to lower classification. The physical–chemical parameters used to calculate the WQI can be found in Table 1 of the supplementary information

Table 1 Parameters and the respective scores used to calculate the integrated index that includes the three LOE of freshwater parameters (WQI, AAMBI and phytotoxicity) assessed in the study area

Rating categories	Score
1—WQI	
Excellent (range value 91–100)	4.5–5.0
God (range value 71 ≤ 91)	3.5–4.5
Medium (range value 51 ≤ 71)	2.5–3.5
Bad (range value 26 ≤ 51)	1.2–2.5
Very Bad (< 26)	0–1.2
2—Phytotoxicity	
Growth enhanced until 30% from control or inhibition until 20% from control	4.0–5.0
Growth enhanced 31% to 60% from control or inhibition 21% to 40% from control	3.0–4.0
Growth enhanced upper to 60% from control or inhibition 41% to 60% from control	2.0–3.0
Growth inhibition 61% to 80% from control	1.0–2.0
Growth inhibition upper 80% from control	0–1.0
3—AAMBI	
Excellent (value < 121)	4.9–5.00
Very Good (range value 90–120)	3.6–4.9
God (range value 50–89)	2.0–3.5
Regular (range value 36–49)	1.4–1.9
Bad (< 35)	0–1.3

For the ranking categories of each LOE we assigned values from 0 to 5, where 0 represented the lowest score (i.e. least favorable ecosystem conditions) and 5 represents the highest score (i.e., most favorable ecosystem conditions)

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values found in contaminated areas. Turbidity ranged from 0.7 NTU (FT site) to 843 NTU (UA site). Turbidity for GM (276 NTU) and UA sites (843 NTU) were above 100 NTU, the threshold established by Ecuadorian guidelines. High turbidity in GM can be due to the influx of sediment particles due to the constant erosion of riverbanks, quite common in mining areas (Batsaikhan et al. 2017).

Physical–chemical parameters varied among sites. Regarding nitrogenous compounds, the LF site presented the highest values of total ammonia (22.1 mg L^{-1}), ammonium (13.4 mg L^{-1}) and nitrate (1.4 mg L^{-1}). High values of total ammonia can indicate a process of degradation of organic matter and organic contamination, usually associated with microbiological contamination. In solution, ammonia toxicity is caused by NH_3 and $NH4^+$, with NH_3 contributing to greater toxicity to the organisms (Banerjee and Srivastava 2009).The nitrite exceeds the threshold for Ecuadorian guidelines (> 0.2 mg L^{-1}) in LF (1.4 mg L^{-1}). High nitrite concentration can result from incomplete ammonia oxidation (Guo et al. 2016) that can be caused by low DO value found in the LF site (16%). In higher concentrations, nitrites are toxic to aquatic organisms (LC50 value of 0.5 mg L^{-1}, calculated from linear regression models) (Kocour Kroupová et al. 2018). The LF site also had the highest fecal coliform load among all sites (100,000 CFU/100 ml).

The UA and LF sites presented highest concentrations of phosphates (0.2 mg L^{-1} and 0.5 mg L^{-1}), which can be caused by nutrient input from point and diffuse sources related to agricultural practices and urban population in these areas. High phosphorus concentrations leads to eutrophication and depletion of DO concentrations (Gupta et al. 2017) and causes imbalances at the base of food webs that impair ecosystem function and community structure (Colborne et al. 2019).

The concentrations of most of the major ions (Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^- and SO_4^{2-}) in all sites were below the thresholds for Ecuadorian and Canadian guidelines (Table 1, supplementary information). However, some ions such as Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^- were found in concentrations 10 to 50-fold higher in GM, UA and LF sites when compared to FT sites, which indicates that different contamination sources can modify water parameters even when below allowed limits. These results indicate that the thresholds need to be revised for Andes-Amazonia rivers.

LOE 2: In water, phytotoxicity was detected only at the FF site. Signals of toxicity were detected at sites FT, FF, GM, UA and LF, both in root and root growth (Fig. 3a), which may indicate the excess of organic matter and metal contamination at these locations (Belz et al. 2018). In sediment, toxicity was detected at FF, GM, UA and LF sites. This effect was probably caused by sewage discharged or complex mixtures of contaminants present in sediments (Marsalek et al. 1999). Sediments are a reservoir of contaminants, which can be a source of chronic contamination (Chapman 1990) due to this the phytotoxicity can be observed mainly in in that matrix. FF, UA and LF sites also showed signals of toxicity, with high root growth in sediment samples. Besides that, germination in sediment was the lowest in the LF site (62%) (Fig. 3b).

At high contaminants concentrations, *L. sativa* germination inhibition would be expected, while growth stimulation would be expected at low concentrations (Belz et al. 2018). Phytotoxicity tests had similar results compared to Capparelli et al. (2020) for the same collection sites, although in

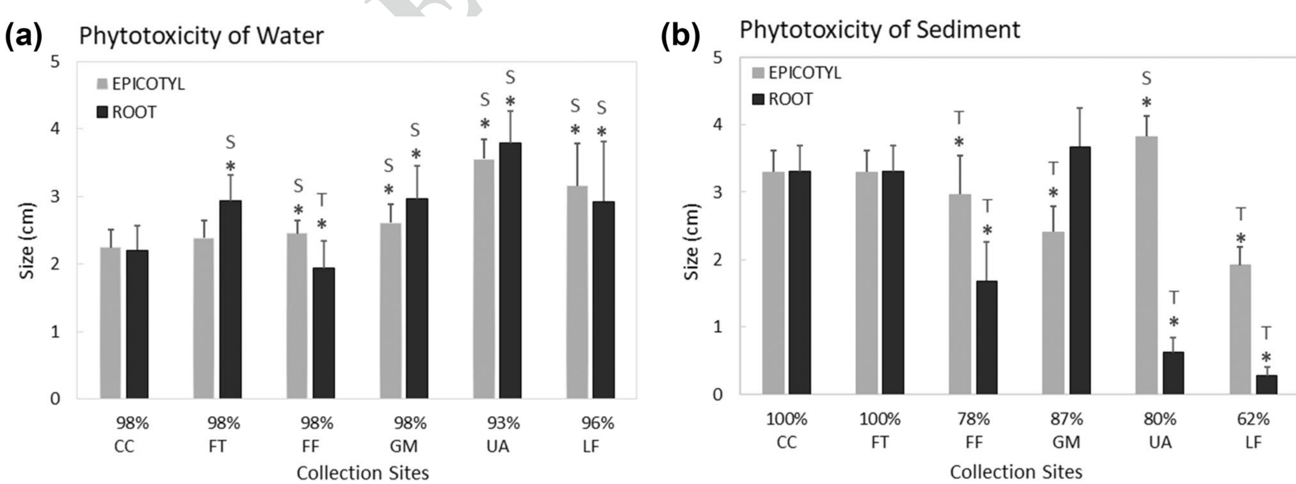

Fig. 3 Results of water (**a**) and sediment (**b**) phytotoxicity tests using seeds of *Lactuca sativa*. Phytotoxicity was evaluated by germination % and by comparing epicotyl and root size exposed to water and sediments taken from sampling sites in comparison with control using Student's t-test. Bars show the mean ± standard error (n = 15) in root or epicolty growth. * Indicates significant mean differences ($p < 0.05$), *T* indicates toxicity (i.e. growth inhibition in the samples), *S* indicates signals of toxic (i.e. growth increased in the samples). The control sample (CC) in water analysis was distilled water and in sediment analysis was the sediments of FT site

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our study, the sediment matrix caused greater toxicity than the water matrix. The high growth of epicotyl, compared to the root growth, in areas such as UA, LF and GM, may indicate the presence of high amounts of organic matter and nutrients, and can also be considered as an indicator of contamination (Lyu et al. 2018).

LOE 3: A total of 132 individuals from 13 families were found. The aquatic macroinvertebrates community was high in number of individuals and more diverse in sites FT and FF, with 12 and 35 individuals, distributed in 5 and 6 families, respectively (Table 2, Table 2 supplementary information). Chironomidae and Glossosomatidae were the only families found in LF and UA sites. These families are tolerant to high organic and inorganic contamination (Roldán-pérez 2016), such as the one caused by the presence of untreated sewage and landfill leachate. Overall, high concentration of metals (Wang et al. 2019), elevated levels of turbidity, TDS, electrical conductivity, nitrate and phosphates are known to affect the diversity and abundance of macroinvertebrate communities (Regina et al. 2010). Moreover, the low DO found in LF (16%) and UA sites (64%) causes oxygen depletion that may also affect the diversity of organisms (Grall and Glemarec 1997).

No macroinvertebrates were found at the GM site. The complete absence of macroinvertebrates in a site could be an indicative of extreme contamination or high degradation of the aquatic ecosystem. It has been documented that mining activities impact the benthic macroinvertebrate communities (Costas et al. 2018), increasing metal contamination, turbidity and color as the result of modifications in the landscape and in the river hydrology caused by mining machines (Luoma et al. 2010). Indeed, high values of turbidity and color were registered in this study and high concentrations of Cd, Cu, Pb were registered for the same GM site in a previous study (Capparelli et al. 2020). On the other hand, FF and FT sites had the highest number of macroinvertebrates families as well as abundances of individuals. At the FT site, families sensitive to contamination (e.g. Leptophlebiidae, Perlidae, Corydalidae) and indicators of clean water were found (Roldán-pérez 2016). FF site harboured families characterized to have intermediate (Elmidae, Hydropsychidae) and high (Chironomidae) resistance to contamination (Table 2).

Integrated LOE: We combined three LOE to evaluate environmental impacts in sites located near contamination sources in the upper Napo River tributaries. Our results showed that FT site had the highest score (12.10), meaning better quality of the aquatic ecosystem and that the UA (6.15) and LF (5.71) sites were the most affected by environmental contamination (Table 3). The score differences between FT and the other sites were 30%–53% lower. Each of these LOE indicates a different way of assessing water quality, but they reflected a similar trend when combined into a single metric. Based on previous knowledge by metal contamination of the study area, we expected water quality from sampling sites to rank in the following order: FT > FF > UA > GM > LF (Capparelli et al. 2020). However, using our integrated LOE assessment, the site's rank resulted in FT > FF > GM > UA > LF. The main difference in relation to our initial hypothesis is the ranking position of UA and GM sites, which can be due to the lack of a LOE for chemical metal analysis. This was somehow reflected by the absence of macroinvertebrates in the GM site, although specific LOE for certain contamination sources would have the resolution to detect more subtle differences between contamination types.

Table 2 Scores of benthic macroinvertebrate families according to the AAMBI index in each collection site

Families	Collection sites				
	FT	FF	GM	UA	LF
Chironomidae	2	2		2	2
Limoniidae	4				
Glossosomatidae					2
Ptilodactylidae	5				
Psphenidae	5				
Naucoridae		5			
Hydropsichidae	5	5			
Leptoceridae	8				
Elmidae	5	5			
Leptohyphidae	7	7			
Corydalidae	9				
Perlidae	10				
Leptophlebiidae	10				
Abundance	35	35	0	19	66
Total Families	11	5	0	1	2
AAMBI	70	24	0	2	4

The abundance of individuals (i.e. the number of individuals of each species) is also reported for each site

Table 3 Integrative matrix analysis of three lines of evidence: Water quality index (WQI), Phytotoxicity and the Andean-Amazon Biotic Index (AAMBI)

Collection sites	Lines of evidence			Total
	WQI	Phytotoxicity	AAMBI	
FT	4.47	4.83	2.8	12.10
FF	3.43	4.1	0.96	8.49
GM	3.37	4.07	0	7.44
UA	2.5	3.57	0.08	6.15
LF	2.15	3.19	0.36	5.70

The scores of each LOE range from 0 to 5; Total scores are the sum of the scores of each LOE

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The LF site was the most impacted site according to the WQI and Phytotoxicity tests. The sanitary landfill where samples were taken has already reached its upper capacity and the drainage system has exceeded its maximum limit, producing a leachate that flows directly into a small nearby stream. The UA sites showed also high degradation, with scores similar to those found at LF sites in our integrated index. Due to the lack of proper water treatment, discharge of wastewater is a chronic source of contamination to Amazonian rivers. The continuous and direct release of domestic effluents into the aquatic environment may eventually lead to the accumulation of contaminants in water and sediment, changing environmental quality (Rocha et al. 2017).

The GM site presented intermediate values to the WQI, with medium quality and signal of toxicity in water tests (root and epicotyl). In the sediment tests it showed toxicity in the epicotyl. Although GM has historically been associated with environmental degradation, the WQI and the phytotoxicity tests alone do not seem to be adequate for identifying this type of impact. In this case chemical analysis of metals would be extremely important. The AAMBI indicates that the GM site is not favorable to macroinvertebrate survival. The inclusion of AAMBI in our integrated index lowered the score that otherwise would be higher for the GM site. In this study, the AAMBI was the LOE which better detected mining effects on water quality. The absence of macroinvertebrates in the GM site should be interpreted as a warning signal, as the pervasive impacts caused by mining activities can be long-term and widespread (Gómez-Barris 2018; Mora et al. 2019). As mining affects several characteristics of an ecosystem, multiple LOE are required to detect the impacts of this activity, including complementary LOE not used in this study, such chemical analysis of the presence and concentration of metals in sediments that may be the destination of the materials from mining discharges. We reinforce the need of further studies on mining impacts, as mining activities tend to increase in the coming years (Roy et al. 2018; Ecuadorian Ministry of Mines 2017).

The FF site showed medium water quality and no phytotoxicity, but AAMBI indicated signals of environmental contamination, most likely due to the presence of metals originating from fish feed, fertilizers, or active principles constituents of chemical compounds applied for different uses (Forster et al. 2003; Tacon et al. 2009). This site is located in rivers that receive aquaculture effluent discharges of about 20 fish farming pools of about 0.2 ha each. The FT location was found to be the least degraded by environmental contamination. This site is located in rivers that flow directly from the Colonso–Chalupas Biological Reserve (CCBR), with few threats in its vicinity, only with sparse population settlements. Although these populations have no basic sanitation treatment, no detectable impacts in the quality of the aquatic ecosystem has been found. Physical–chemical parameters in FT sites are well below those reported to the Napo basin (Alexiades et al. 2019) and are close to parameters appropriate for drinking water.

PCA analysis showed a spatial separation between sampling sites. PC1 explained 58.8% of the variance and separated all sites according to the selected variables. PC2 explained 25.7% of the variance; sites were separated by the phytotoxicity parameters epicotyl growth and seed germination in the sediments and root growth in water (Fig. 4, Table 3 of supplementary information). When taken only metal contamination into account, LF sites also did not cluster with other sites and UA grouped with FT sites (Capparelli et al. 2020). However, in our study UA and FT were separated by PC1. The separation of sites indicated that they are affected by different contamination sources and that the identification of the impact of contamination sources may not be easy to detect without appropriate LOE.

Our study confirmed that multiple threats have significant impacts on aquatic ecosystems at the Andes-Amazonia ecotone. The combination of water quality indices and benthic macroinvertebrates with phytotoxicity allowed us to draw more complete conclusions about environmental impacts. Ecuadorian environmental legislation for the preservation of aquatic life (TULSMA 2015) considers only physical–chemical parameters, including metals, pesticides, and surfactants, which alone are not sufficient to indicate the degree of environmental contamination. We showed that biological indicators such as toxicity essays and macroinvertebrate indices are low-cost and sensitive for detecting environmental impacts. Therefore, assessments including multiple LOE, such as those used in this study, appear to be useful and accessible tools for constant and long-term monitoring of fragile aquatic ecosystems, which may help to detect further deterioration. Complementarily, we suggest including the chemical evaluation of the sediment as LOE in future investigations.

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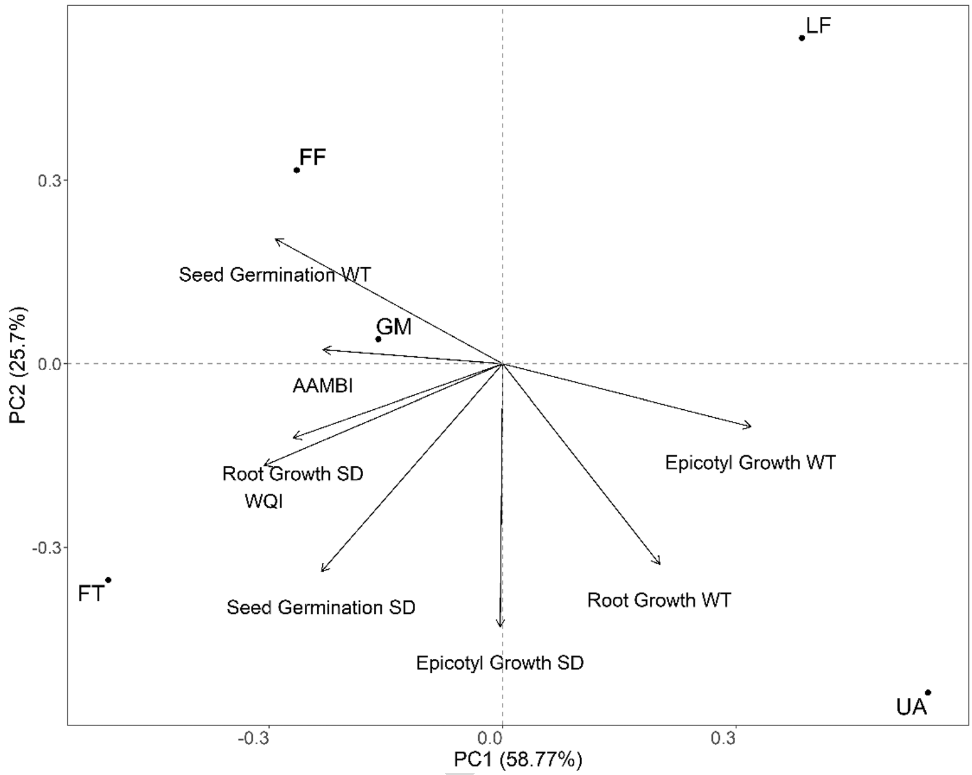

Fig. 4 The dimensional space is determined by the two first PCA axes, summarizing the main environmental gradients formed by each LOE and by parameters derived from the phytotoxicity tests with *L. sativa* in water (WT) and in sediments (SD) matrices. Sampling sites are few threats (FT), fish farming (FF), golden mining (GM), urban areas (UA) and landfill (LF)

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could influence the present investigation.

References

Alexiades AV, Encalada AC, Lessmann J, Guayasamin JM (2019) Spatial prediction of stream physicochemical parameters for the Napo River Basin. Ecuador J Freshw Ecol 34(1):247–261

Altenburger R, Ait-aissa S, Antczak P, Backhaus T, Barceló D, Seiler T, Brion F, Busch W, Chipman K, López M, Alda D, Aragão GD, Escher BI, Falciani F, Faust M, Focks A, Hilscherova K, Hollender J, Hollert H, Jäger F, Jahnke A, Kortenkamp A, Krauss M, Lemkine GF, Munthe J, Neumann S, Schymanski EL, Scrimshaw M, Segner H, Slobodnik J, Smedes F, Kughathas S, Teodorovic I, Tindall AJ, Tollefsen KE, Walz K-H, Williams TD, Van den Brink PJ, van Gils J, Vrana B, Zhang X, Brack W (2015) Future water quality monitoring—Adapting tools to deal with mixtures of pollutants in water resource management. Sci Total Environ 512–513:540–551. https://doi.org/10.1016/j.scitotenv.2014.12.057

APHA (1998) Standard methods for the examination of water and wastewater. American Public Health Association, 25th edn, pp. 1–101. Washington, DC, Centennial. ISBN 9780875532356.

Backhaus T, Brack W, Van den Brink PJ, Deutschmann B, Hollert H, Posthuma L, Segner H, Seiler TB, Teodorovic I, Focks A (2019) Assessing the ecological impact of chemical pollution on aquatic ecosystems requires the systematic exploration and evaluation of four lines of evidence. Environ Sci Eur. https://doi.org/10.1186/s12302-019-0276-z

Batsaikhan B, Kwon J-S, Kim K-H, Lee Y-J, Lee J-H, Badarch M, Yun S-T (2017) Hydrochemical evaluation of the influences of mining activities on river water chemistry in central northern Mongolia. Environ Sci Pollut Res 24(2):2019–2034. https://doi.org/10.1007/s11356-016-7895-3

Banerjee T, Srivastava RK (2009) Application of water quality index for assessment of surface water quality surrounding integrated industrial estate-Pantnagar Tirthankar Banerjee and Rajeev Kumar Srivastava. 2041–2054. https://doi.org/10.2166/wst.2009.537

Belz RG, Patama M, Sinkkonen A (2018) Low doses of six toxicants change plant size distribution in dense populations of Lactuca sativa. Sci Total Environ 631:510–523

Burton GA Jr (2002) Sediment quality criteria in use around the world. Limnology 3(2):65–76

Canadian Council of Ministers of the Environment CCME (2002) Canadian environmental quality guidelines (Vol. 2). Canadian Council of Ministers of the Environment

Capparelli MV, Moulatlet GM, de Souza Abessa DM, Lucas-Solis O, Rosero B, Galarza E, Tuba D, Carpintero N, Ochoa-Herrera V, Cipriani-Avila I (2020) An integrative approach to identify the impacts of multiple metal contamination sources on the Eastern Andean foothills of the Ecuadorian Amazonia. Sci Total Environ 709:136088. https://doi.org/10.1016/j.scitotenv.2019.136088

Chapman PM (1990) The sediment quality triad approach to determining pollution-induced degradation. Sci Total Environ 97:815–825

Chapman PM, McDonald BG, Lawrence GS (2002) Weight-of-evidence issues and frameworks for sediment quality (and other) assessments. Hum Ecol Risk Assess 8(7):1489–1515. https://doi.org/10.1080/20028091057457

Costas N, Pardo I, Méndez-Fernández L, Martínez-Madrid M, Rodríguez P (2018) Sensitivity of macroinvertebrate indicator taxa to metal gradients in mining areas in Northern Spain. Ecol Indic 93:207–218

Colborne SF, Maguire TJ, Mayer B, Nightingale M, Enns GE, Fisk AT, Drouillard KG, Mohamed MN, Weisener CG, Wellen C, Mundle SOC (2019) Water and sediment as sources of phosphate in aquatic ecosystems: The Detroit River and its role in the Laurentian Great Lakes. Sci Total Environ 647:1594–1603. https://doi.org/10.1016/j.scitotenv.2018.0

Cox BA (2003) A review of currently available in-stream water-quality models and their applicability for simulating dissolved oxygen in lowland rivers. Sci Total Environ 314–316:335–377. https://doi.org/10.1016/S0048-9697(03)00063-9

Dagnino A, Sforzini S, Donderor F, Fenoglio S, Bona E, Jensen J, Viarengo A (2008) A weight-of-evidence approach for the integration of environmental “triad” data to assess ecological risk and biological vulnerability. Integr Environ Assess Manag 4(3):314–326

Ecuadorian Ministry of Mines (2017) Concessions. Retrieved from http//:geo.controlminero.gob.ec

Encalada AC, Flecker AS, Poff NL, Suárez E, Herrera-R GA, Ríos-Touma B, Jumani S, Larson EI, Anderson EP (2019a) A global perspective on tropical montane rivers. Science 365(6458):1124–1129

Encalada AC, Guayasamin JM, Suárez E, Mena CF, Lessmann J, Sampedro C, Martínez P, Ochoa-Herrera V, Swing K, Celinacak M, Schreckinger J, Vieira J, Tapia A, Serrano C, Barragán K, Andrade S, Alexiades A, Troya MJ (2019b) Los ríos de las cuencas Andino-Amazónicas: Herramientas y guía de invertebrados para el diseño de programas de monitoreo

Finer M, Jenkins CN, Pimm SL, Keane B, Ross C (2008) Oil and gas projects in the Western Amazon: Threats to wilderness, biodiversity, and indigenous peoples. PLoS ONE. https://doi.org/10.1371/journal.pone.0002932

Flores M, Lopes U, Panuncio M, Riveros JC, Rodrigues S, Valenzuela S, Arancibia D, Bara-neto P (2010) WWF’s Living Amazon Initiative

Forster IP, Dominy W, Obaldo L, Tacon AGJ (2003) Rendered meat and bone meals as ingredients of diets for shrimp Litopenaeus vannamei (Boone, 1931). Aquaculture 219(1–4):655–670

Gómez-Barris M (2018) Review of la Amazonía Minada: Minería a gran escala y conflictos en el sur del Ecuador. In: European Review of Latin American and Caribbean Studies | Revista Europea de Estudios Latinoamericanos y del Caribe. https://doi.org/10.32992/erlacs.10427

Grall J, Glémarec M (1997) Using biotic indices to estimate macrobenthic community perturbations in the Bay of Brest. Estuar Coastal Shelf Sci 44:43–53

Grill G, Lehner B, Thieme M, Geenen B, Tickner D, Antonelli F, Babu S, Borrelli P, Cheng L, Crochetiere H, Ehalt Macedo H, Filgueiras R, Goichot M, Higgins J, Hogan Z, Lip B, McClain ME, Meng J, Mulligan M, Nilsson C, Olden JD, Opperman JJ, Petry P, Reidy Liermann C, Sáenz L, Salinas-Rodríguez S, Schelle P, Schmitt RJP, Snider J, Tan F, Tockner K, Valdujo PH, van Soesbergen A, Zarfl C (2019) Mapping the world’s free-flowing rivers. Nature 569(7755):215–221. https://doi.org/10.1038/s41586-019-1111-9

Gupta N, Pandey P, Hussain J (2017) Effect of physicochemical and biological parameters on the quality of river water of Narmada, Madhya Pradesh. India Water Sci 31(1):11–23. https://doi.org/10.1016/j.wsj.2017.03.002

Guo Y, He L-L, Zhao D-X, Gong L-D, Liu C, Yang Z-Z (2016) How does ammonia bind to the oxygen-evolving complex in the S2 state of photosynthetic water oxidation? Theoretical support and implications for the W1 substitution mechanism. Phys Chem Chem Phys 18(46):31551–31565

Hering D, Feld CK, Moog O, Ofenbo T (2006) Cook book for the development of a Multimetric Index for biological condition of aquatic ecosystems: experiences from the European AQEM and STAR projects and related initiatives. https://doi.org/10.1007/s10750-006-0087-2

Hoekstra NT, Bosker T, Lantinga T (2002) Effects of cattle dung from farms with different feeding strat- egies on germination and initial root growth of cress (*Lepidium sativum* L.). Agric Ecosyst Environ 93:189–196

Hussan A, Gon T (2016) Common problems in aquaculture and their preventive measures. Aquac Times J 2(5):6–9

INEC (2010) Fasiculo provincial Napo: 0–7.

Isch E (2011) Contaminación de las aguas y políticas para enfrentarla 52. http://www.camaren.org/documents/contaminacion.pdf

Josse C, Cuesta F, Navarro G, Barrena V, Cabrera E, Chacón-Moreno E, Ferreira W, Peralvo M, Saito J, Tovar A (2009) Ecosistemas de los Andes del norte y centro. Bolivia, Colombia, Ecuador, Perú y Venezuela

Kocour Kroupová H, Valentová O, Svobodová Z, Šauer P, Máchová J (2018) Toxic effects of nitrite on freshwater organisms: a review. Rev Aquac 10(3):525–542

Koçer MAT, Sevgili H (2014) Parameters selection for water quality index in the assessment of the environmental impacts of land-based trout farms. J Ind Ecol 36:672–681. https://doi.org/10.1016/j.ecolind.2013.09.034

Lessmann J, Troya MJ, Flecker AS, Funk WC, Guayasamin JM, Ochoa-Herrera V, Poff NL, Suárez E, Encalada AC (2019) Validating anthropogenic threat maps as a tool for assessing river ecological integrity in Andean-Amazon basins. PeerJ 7:e8060. https://doi.org/10.7717/peerj.8060

Luoma SN, Cain DJ, Rainbow PS (2010) Calibrating biomonitors to ecological disturbance: a new technique for explaining metal effects in natural waters. Integr Environ Assess Manag Int J 6(2):199–209

Lyu J, Park J, Kumar Pandey L, Choi S, Lee H, De Saeger J et al (2018) Testing the toxicity of metals, phenol, effluents, and receiving waters by root elongation in *Lactuca sativa* L. Ecotoxicol Environ Saf 149:225–232. https://doi.org/10.1016/j.ecoenv.2017.11.006

Marsalek J, Rochfort Q, Brownlee B, Mayer T, Servos M (1999) An exploratory study of urban runoff toxicity. Water Sci Technol 39(12):33–39

McClain ME, Naiman RJ (2008) Andean influences on the biogeochemistry and ecology of the Amazon River. BioScience 58(4):325–338

Melcher AH, Bakken TH, Friedrich T, Greimel F, Humer N (2016) Drawing together multiple lines of evidence from assessment studies of hydropeaking pressures in impacted rivers. December. https://doi.org/10.1086/690295

Moquet JS, Guyot JL, Crave A, Viers J, Filizola N, Martinez JM, Oliveira TC, Sánchez LSH, Lagane C, Casimiro WSL, Noriega L, Pombosa R (2016) Amazon River dissolved load: temporal dynamics and annual budget from the Andes to the ocean. Environ Sci Pollut Res 23(12):11405–11429. https://doi.org/10.1007/s11356-015-5503-6

Mora A, Jumbo-Flores D, González-Merizalde M, Bermeo-Flores SA, Alvarez-Figueroa P, Mahlknecht J, Hernández-Antonio A (2019) Heavy metal enrichment factors in fluvial sediments of an amazonian basin impacted by gold mining. Bull Environ Contam Toxicol 102(2):210–217. https://doi.org/10.1007/s00128-019-02545-w

Nong X, Shao D, Zhong H, Liang J (2020) Evaluation of water quality in the South-to-North Water Diversion Project of China using the

Journal : Large 128 | Article No : 3089 | Pages : 10 | MS Code : 3089 | Dispatch : 21-1-2021

water quality index (WQI) method. Water Res 128:115781. https://doi.org/10.1016/j.watres.2020.115781

Noori R, Berndtsson R, Hosseinzadeh M, Adamowski JF, Abyaneh MR (2018) A critical review on the application of the National Sanitation Foundation Water Quality Index. Environ Pollut. https://doi.org/10.1016/j.envpol.2018.10.076

OECD TG 208 (2006) Terrestrial plant test: seedling emergence and seedling growth test. OECD Guidelines for the Testing of Chemicals, Section, 2. OECD Publishing, Paris, July.

Regina S, Couceiro M, Hamada N, Forsberg BR, Padovesi-fonseca C (2010) Effects of anthropogenic silt on aquatic macroinvertebrates and abiotic variables in streams in the Brazilian Amazon: 89–103. https://doi.org/10.1007/s11368-009-0148-z

Rocha CA, Sousa FW, Zanella ME, Oliveira AG, Nascimento RF, Souza OV, Cajazeiras IM, Lima J, Cavalcante RM (2017) Environmental quality assessment in areas used for physical activity and recreation in a city affected by intense urban expansion (Fortaleza-CE, Brazil): Implications for public health policy. Expos Health 9(3):169–182

Roldán-Pérez G (2016) Los macroinvertebrados como bioindicadores de la calidad del agua: cuatro décadas de desarrollo en Colombia y Latinoamerica. Revista de la Academia Colombiana de Ciencias Exactas. Físicas y Naturales 40(155):254–274

Roy BA, Zorrilla M, Endara L, Thomas DC, Vandegrift R, Rubenstein JM, Policha T, Ríos-Touma B, Read M (2018) New mining concessions could severely decrease biodiversity and ecosystem services in Ecuador. Trop Conserv Sci 11:1940082918780427

Santos R, Joyeux A, Besnard A, Blanchard C, Halkett C, Bony S, Sanchez W, Devaux A (2017) An integrative approach to assess ecological risks of surface water contamination for fish populations. Environ Pollut 220:588–596

Stoddard JL, Herlihy AT, Peck DV, Hughes RM, Whittier TR, Tarquinio E, Stoddard JL, Herlihy AT, Peck DV, Whittier TR (2008) A process for creating multimetric indices for large-scale aquatic surveys. J North Am Benthol Soc 27(4):878–891. https://doi.org/10.1899/08-053.1

Tacon AG, Metian M, Hasan MR (2009) Feed ingredients and fertilizers for farmed aquatic animals: sources and composition (No. 540). Food and Agriculture Organization of the United Nations (FAO)

Taylor P, Smith EP, Lipkovich I, Ye K, Smith E P, Lipkovich I, Ye K (2010) Weight-of-Evidence (WOE): quantitative estimation of probability of impairment for individual and multiple lines of evidence. January 2015, 37–41. https://doi.org/10.1080/20028091057493

TULSMA (2015) Edición Especial No 387 Registro Oficial – Edición Especial No 387. Registro Oficial 097:6–26

Ustaoğlu F, Tepe Y, Taş B (2020) Assessment of stream quality and health risk in a subtropical Turkey river system: a combined approach using statistical analysis and water quality index. Ecol Indic 113:105815. https://doi.org/10.1016/j.ecolind.2019.105815

U.S.EPA (1996) Ecological Effects Test Guidelines Seed Germination/Root Elongation Toxicity Test. *Test*, April. EPA 712-C-96-154

Wang X, Su P, Lin Q, Song J, Sun H, Cheng D, Wang S, Peng J, Fu J (2019) Distribution , assessment and coupling relationship of heavy metals and macroinvertebrates in sediments of the Weihe River Basin. Sustainable

Wright JF, Moss D, Armitage PD, Furse MT (1984) A preliminary classification of running-water sites in Great Britain based on macro-invertebrate species and the prediction of community type using environmental data: 221–256.

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