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## Integrating multiple lines of evidence to assess freshwater ecosystem health in a tropical river basin<sup>☆</sup>

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### ABSTRACT

Degradation of freshwater ecosystems by uncontrolled human activities is a growing concern in the tropics. In this regard, we aimed at testing an integrative framework based on the IFEQ index to assess freshwater ecosystem health of river basins impacted by intense livestock and agricultural activities, using the Muchacho River Basin (MRB) as a case study. The IFEQ combine multiple lines of evidence such as riverine hydromorphological analysis (LOE 1), physicochemical characterization using ions and pesticides (LOE 2), aquatic macroinvertebrate monitoring (LOE 3), and phytotoxicological essays with *L. sativa* (LOE 4). Overall, results showed an important reduction in streamflow and an elevated increase in ion concentrations along the MRB caused by deforestation and erosion linked to agricultural and livestock activities. Impacts of the high ion concentrations were evidenced in macroinvertebrate communities as pollution-tolerant families, associated with high conductivity levels, represented 92 % of the total abundance. Pollution produced by organophosphate pesticides (OPPs) was critical in the whole MRB, showing levels that exceeded 270-fold maximum threshold for malathion and 30-fold for parathion, the latter banned in Ecuador. OPPs concentrations were related to low germination percentages of *Lactuca sativa* in sediment phytotoxicity tests. The IEFQ index ranged from 44.4 to 25.6, indicating that freshwater ecosystem conditions were “bad” at the headwaters of the MRB and “critical” along the lowest reaches. Our results show strong evidence that intense agricultural and livestock activities generated significant impacts on the aquatic ecosystem of the MRB. This integrative approach better explains the cumulative effects of human impacts, and should be replicated in other basins with similar conditions to help decision-makers and concerned inhabitants generate adequate policies and strategies to mitigate the degradation of freshwater ecosystems.

### 1. Introduction

Uncontrolled human activities such as unplanned urbanization, random use of agrochemicals, extensive livestock farming, and improper disposal of wastewater, decrease water quality and affect the ecological health of river basins (Bashir et al., 2020; Wu et al., 2017). In this regard, comprehensive water quality assessment has become an important tool to develop and implement strategies to safeguard freshwater ecosystems (Grizzetti et al., 2017). The use of multiple lines of evidences (LOEs) has

been widely recommended to assess water quality, as this integrative method gives a wider picture of freshwater ecosystem health that otherwise could not be done by isolated approaches (Altenburger et al., 2019; Backhaus et al., 2019; Buchwalter et al., 2017; Chapman et al., 2016; Merrington et al., 2014; Palma et al., 2018; Reyjol et al., 2014). For instance, physicochemical endpoints provide information on concentrations of chemical stressors in the aquatic ecosystem, but they do not describe how biological communities are affected by them (Posthuma et al., 2019; Serpa et al., 2014). On the other hand, field-based

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biomonitoring using macroinvertebrates gives an idea of pollutant effects on ecological health of river basins (structure and functioning of aquatic communities) without precisely describing the chemical stressors (Backhaus et al., 2019; Shiji et al., 2016).

Physicochemical characterization coupled with macroinvertebrates monitoring generates meaningful results in order to understand freshwater ecosystem health, however, its outcomes cannot reflect the bioavailability of pollutants (Montvydienė et al., 2007; Wang et al., 2001). To solve this limitation, ecotoxicological essays have been integrated into water quality assessment to detect the potential effect of pollutants on the biota (Fabbrocini et al., 2010; Moiseenko et al., 2008). A commonly used ecotoxicological tool is the phytotoxicity essay with *Lactuca sativa* as it is simple, quick, reliable, and cost-effective (Caparelli et al., 2020). Non-chemical stressors, such as riverine hydrological and morphological changes, also play an important role in freshwater ecosystem health (Backhaus et al., 2019). Variables such as streamflow and channel width variability, instream modifications, vegetation cover, deforestation and erosion strongly influence water chemistry, and hence structure and diversity of aquatic communities (Gebler et al., 2018; Palma et al., 2018; Stefanidis et al., 2019). In fact, the EU Water Framework Directive and several authors suggested considering riverine hydromorphology as a line of evidence to assess water quality (Bogardi et al., 2020; van Gils et al., 2019).

In the tropics, not enough studies combining multiple lines of

evidence have been reported despite having several problems related to the degradation of water quality and aquatic ecosystems. Historically, freshwaters in Western Ecuador have been impacted by intense livestock and agricultural activities (Salmoral et al., 2018). It is estimated that three thirds of the total surface of the region has been deforested to satisfy the land demand for agricultural and livestock uses (MAAE, 2020). In spite of this, most water quality studies in the region (e.g., Aveiga et al., 2019; Barahona and Tapia, 2010; Lucas and Carreño, 2018; Quiroz et al., 2017) focus solely on classical physicochemical parameters without considering other chemical stressors, such as ions or pesticides, the main pollutants released by livestock and agricultural activities. Furthermore, the few studies that combined multiple LOEs (e.g., Alvarez-Mieles et al., 2013; Damanik-Ambarita et al., 2018, 2016a; Nguyen et al., 2017) did not consider ecotoxicological components, and their physicochemical results were not adequately contrasted with national or international water quality regulations.

Given the lack of comprehensive documentation on aquatic ecosystem degradation in the tropics, and in particular in western Ecuador, we aimed at testing an integrative framework to assess freshwater ecosystem health of river basins impacted by intense livestock and agricultural activities, using the Muchacho River Basin (MRB) as a case study. The framework combined multiple lines of evidence such as riverine hydromorphological analysis (LOE 1), physicochemical characterization including ions and pesticides (LOE 2), aquatic

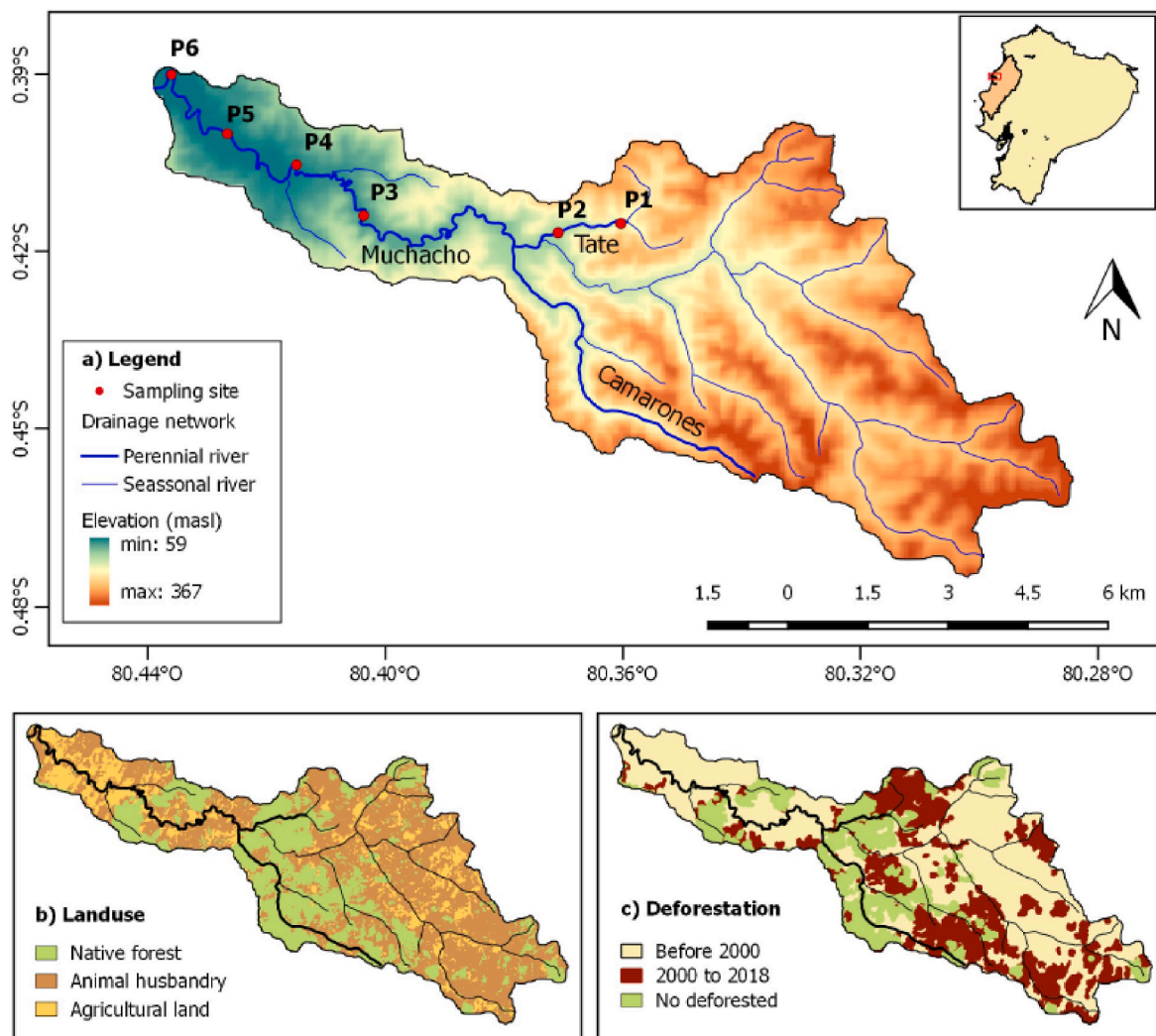


Fig. 1. Location of the Muchacho river basin in western Ecuador. (a) Topography, drainage and sampling sites. (b) Land Use, and (c) deforestation. Data was obtained from Ministerio del Ambiente y Agua del Ecuador (MAAE, 2020).

macroinvertebrates monitoring (LOE 3), and phytotoxicological essays with *L. sativa* (LOE 4). Each LOE was summarized using numerical metrics and statistical analysis frequently used in freshwater ecosystem assessments. We found the framework provides a suitable characterization of cumulative effects of human activities on aquatic ecosystems, including the reduction of both water quality and quantity along a representative tropical river basin. Also, our outcomes give relevant insights to decision-makers and other stakeholders to generate adequate policies and strategies to mitigate the degradation of freshwater ecosystems.

## 2. Materials and methods

### 2.1. Study area

The MRB is located at the transition between northern humid (Mache-Chindul) and southern dry (Chongón-Colonche) tropical forests in the Pacific coast of Manabí province, western Ecuador (Fig. 1a). It drains an area of about 61.9 km<sup>2</sup> along 59 to 367 masl. The drainage network includes three perennial (Tate, Camarones, and Muchacho) and eleven seasonal rivers. Its climate is sub-humid with an average annual rainfall of 600 mm that obeys a unimodal regimen with maximum values in March. Temperature ranges annually between 23 and 31 °C, and relative humidity averages 79% (Cadena et al., 2012).

The basin presents a population of about 412 people and their main economic activities are animal husbandry and cultivation of watermelon, cocoa, and maize (Río Muchacho Organic Farm, 2019). According to the Ministry of the Environment and Water of Ecuador, livestock and agricultural lands cover 76% of the MBR, while the difference is covered by native forests (Fig. 1b) (MAAE, 2020). Most of the forest is concentrated at the center of the MRB, along the Tate and Camarones river banks. However, during the last decades, deforestation in the latter areas increased at alarming rates (Río Muchacho Organic Farm, 2019). In fact, since 2000 about 10.3 km<sup>2</sup> (16.6%) of the basin surface area have been deforested and dedicated to agricultural and livestock activities (Fig. 1c).

### 2.2. Sampling

We established six sampling sites along the MRB based on the Critical Sampling Points (CSP) methodology used in agricultural basins (Strobl et al., 2006b, 2006a). Sampling sites were distributed along an altitude gradient (~124 m) where significant biases by natural variations (i.e., climate, topography) were not generated (Nguyen et al., 2017; Río Muchacho Organic Farm, 2019). Sites P1 and P2 were located at the headwater Tate River, upstream of most human activities (less threatened). The remaining four sites (P3 to P6) were distributed along the middle and lower reaches, near agricultural and livestock activities (Fig. 1a). Field sampling was carried out in January 2020 (end of dry season) and during the previous two weeks no precipitation events were recorded (INAMHI, 2020). For each site, surface water, sediment, and macroinvertebrates samples were collected.

#### 2.2.1. Water and sediment sampling

Three superficial water samples (1 L each) were collected at the center of the stream channel (depth 10 cm), following NTE INEN 2176 recommendations (INEN, 2013). For physicochemical and microbiological analysis, water samples were conditioned in plastic containers. For pesticide analysis, amber-glass bottles were used. On the other hand, one sediment sample was collected at the riverbank in plastic containers (about 100 g), using a plastic hand trowel. Bottles and containers were rinsed three times with sampling water, labeled, and transported under refrigeration (4 °C) to the laboratory.

#### 2.2.2. Macroinvertebrates sampling

Macroinvertebrates were sampled using the multi-habitat method

described in Gabriels et al. (2010), covering a stretch of 10 m during 5 min with a D-frame dip net (500 µm). We decided to use this sampling method as it is widely recommended to evaluate water quality using macroinvertebrates in Ecuador (Damanik-Ambarita et al., 2016a, 2016b; Galarza et al., 2021; Nguyen et al., 2017; Van Echelpoel et al., 2018). The method considers each microhabitat and substrate present at each site as a subsample. All subsamples were placed in the same container and treated as a composite sample in order to ensure thorough biodiversity assessment at each sampling site.

### 2.3. Riverine hydromorphological data collection

Discharge and flow velocity were measured at each sampling station using the electromagnetic flow-meter HACH FH950. Also, a visual assessment of the riverine hydromorphology was conducted using the quick guide developed by Celi et al. (2018). The evaluation focused on deforestation and erosion levels along the riparian zone, anthropogenic instream modification (e.g., dredging, water extraction, or weirs), and channel width variation (Table S1).

### 2.4. Freshwater ecosystem quality

#### 2.4.1. Physicochemical and microbiological characterization

Water temperature, pH, dissolved oxygen (DO), electrical conductivity (EC), and total dissolved solids (TDS) were measured in-situ using a YSI pro-plus multiparameter instrument. Chemical oxygen demand (COD), turbidity, fecal and total coliforms were analyzed in the laboratory. COD was measured using the Dichromate method (HACH TNT822 vial test) and a HACH DR 1900 spectrophotometer. Turbidity was measured with a HACH TL2300 turbidimeter. Finally, fecal and total coliforms were measured according to Standard Methods (APHA, 2017), satisfying the maximum holding time (less than 24 h).

#### 2.4.2. Ions and organophosphate pesticides

Cations (Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, NH<sub>4</sub><sup>+</sup>) and anions (F<sup>-</sup>, Cl<sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, SO<sub>4</sub><sup>2-</sup>) analyses were carried out using an ion chromatographer Shimadzu (Shodex IC-52 4 E anion and Shodex IC YS-50 cation) following the methodology described by Standard Methods (APHA, 2018).

For organophosphate pesticide (OPP) analysis, water samples were transferred to separating funnels and added 50 g of NaCl, shaking until completely diluted. OPPs were extracted by shaking the samples for 1 min using 15 mL of dichloromethane (three times). Then, the extract was filtered with 3 g sodium sulfate anhydrous and rotaevaporated to 1 mL (Khalili-Zanjani et al., 2008; Montuori et al., 2015). Extracts were analyzed by gas chromatography with the nitrogen-phosphorus detector GC-2014 Shimadzu. Compound identification was carried out by comparing retention times with reference standard mixtures (96–99 % certified purity) of 7 OPPs: Dimethoate, EPN, Malathion, Monocrotophos, Parathion, Sulfotepp, and Tetraethylpyrophosphate. These OPPs were chosen based on their frequent use in western Ecuador (DeKnock et al., 2019; Villegas et al., 2021). The limits of detection (LODs) for organophosphate pesticides ranged between 0.008 and 0.010 µg/L in water samples. The percent recovery of each pesticide was 55–95 % in water.

#### 2.4.3. Water quality regulations

Physicochemical and microbiological parameters, ion and pesticide concentrations were compared to Ecuadorian (MAE, 2015) and American (USEPA, 2017, 1986) regulations for the protection of aquatic life and irrigation. Canadian regulation (CCME, 2002) was also used to contrast ion and OPP concentrations. We decided to compare outcomes of the chemical analyses using international regulations since Ecuadorian water quality regulation is permissible for various parameters.



#### 2.4.4. Macroinvertebrate identification

Collected macroinvertebrates were identified to the lowest practical taxa (family) according to standard taxonomic keys described in Domínguez and Fernández (2009), Darrigran (2013), and Palma (2013). For each sampling site, the status of macroinvertebrate communities was evaluated by total abundance (N), relative abundance (R), and biological diversity using Shannon-Weaver (H') and Gini-Simpson (L') indices (Qureshi et al., 2020; Spellerberg and Fedor, 2003). Shannon-Weaver ranges from 1 (low diversity) to 4.5 (high diversity) and Gini-Simpson varies from 0 (no diversity) to 1 (high diversity).

#### 2.4.5. Phytotoxicity tests

Seed germination, hypocotyl and root elongation of *Lactuca sativa* were tested using water and sediment samples according to the methodology used in Capparelli et al. (2020). Ten seeds were distributed on a filter paper in sterile Petri dishes with 5 mL of water samples. Three replicates were performed for control (distilled water) and each water sample. Subsequently, Petri dishes were covered and left in the dark for 5 days at ambient temperature. On the other hand, 30 g of sediment were placed in a plastic container (about 100 g) and eight seeds were spread on each container. For each sediment sample, three replicate tests were performed according to USEPA (1996) protocol. For this test, the control was a sediment sample from the Río Muchacho Organic Farm which protects part of the riparian forest from anthropogenic pressures.

### 2.5. Numerical and statistical analysis

To assess the quality of the freshwater ecosystem the parameters and indicators analyzed in this study were summarized using various numerical and statistical indices. We considered three main criteria to choose the indices: (i) adapted for tropical conditions, (ii) frequently used in western Ecuador, and (iii) simple, quick, and reliable to implement.

#### 2.5.1. Riverine hydromorphological condition index (LOE 1)

Riverine hydromorphological indicators (level of deforestation, erosion, dredging, water extraction, and channel width variation due to human activities) were numerically reclassified according to the impact on the ecology of the river (Table S1), where 0 represents no impact and 3 describes high impact. The riverine hydromorphological condition index (RHC) was computed as the sum of each indicator, following the recommendation presented in Damanik-Ambarita et al. (2016a) and Keogh et al. (2020).

#### 2.5.2. Physicochemical water quality indices (LOE 2)

Physicochemical water quality of each sampling site was determined according to the National Sanitation Foundation's Water Quality Index (NSF-WQI) and Canadian Council of Ministers of the Environment Water Quality Index (CCME-WQI). Procedure and equations for both NSF-WQI and CCME-WQI are fully described in Kachroud et al. (2019). For this study, CCME-WQI computation was carried out with all analyzed parameters (physicochemical, microbiological, ions, and pesticides), except for those without water quality threshold.

#### 2.5.3. Biological water quality indices (LOE 3)

Biological water quality was assessed through three indices that are commonly used in river basins of Ecuador: Biological Monitoring Working Party modified for Colombia (BMWP) (Damanik-Ambarita et al., 2016b), Average Score Per Taxon (ASPT) (Zamora-Muñoz et al., 1995), and Ephemeroptera Plecoptera and Trichoptera (EPT) (Machado et al., 2018). Note these are the most recommended indices to assess the study region (Damanik-Ambarita et al., 2016b; Nguyen et al., 2017).

#### 2.5.4. Phytotoxicity levels (LOE 4)

To assess the growth of *L. sativa* for each treatment, the plant size (hypocotyl + root) was measured. According to Shapiro-Wilks and

Fligner tests, data did not satisfy the assumption of normality and homoscedasticity, respectively. Thus, plant size data were compared with their respective lab control using non-parametric Wilcoxon tests. Samples were considered toxic when mean plant size had statistically significant differences ( $p < 0.05$ ) below the respective control. Additionally, alterations in germination and normal development of seedlings were analyzed using the germination-root index (GI) which considers the root elongation and germination percentage with respect to lab controls. A complete description of GI was presented in Young et al. (2016).

#### 2.5.5. Integrative framework: the IFEQ index

Integration of multiple lines of evidence was performed based on the recommendations presented in Vollmer et al. (2018). First, the average of the metrics for each LOE was computed. Subsequently, the resulting values were aggregated using the geometric mean, as we present in equation (1).

$$IFEQ = \sqrt[N]{\prod_{i=1}^N \left( \frac{1}{k_i} \sum_{j=1}^{k_i} F_{ij} \right)} \quad (1)$$

Where, IFEQ is the integrative freshwater ecosystem quality index, N describes the number of LOEs (for this study  $N = 4$ ),  $k_i$  represents the number of used metrics for each LOE, and  $F_{ij}$  represents the metrics. Note the used metrics in this study present different scales. Thus, they were set to a 0–100 scale in order to ensure suitable scalability during data aggregation. On the scale, 0 represents “critical freshwater ecosystem condition” and 100 describes “excellent freshwater ecosystem condition” (Table S2).

Additionally, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to summarize the outcomes and determine natural groups based on the (dis)similarities of hydromorphological, physicochemical, biological, and phytotoxicological characteristics. For HCA, we used the Ward algorithm as the agglomeration method (Ward, 1963). Prior to these statistical analyzes, all variables were normalized by setting their sum of squares to one. HCA and PCA were carried out with R project v.4.0.0 (R Core Team, 2019).

## 3. Results and discussion

### 3.1. Riverine hydromorphological condition

Discharge greatly decreased downstream along the MRB. Headwaters discharge (P1, P2) averaged  $0.058 \text{ m}^3/\text{s}$ , while at middle reaches (P3, P4) averaged  $0.033 \text{ m}^3/\text{s}$ . At lower reaches (P5, P6), the streamflow averaged  $0.019 \text{ m}^3/\text{s}$  (Table 1). This behavior has been widely reported in basins that drain small areas with a strong presence of agricultural and livestock intervention (Steinfeld et al., 2006), as is the case of the MRB. In fact, improvised water pumping systems were observed at most sites in the middle and lower reaches (P3 to P6), confirming that water extraction for irrigation and animal husbandry activities is an important driver of streamflow decrease in the MRB. Various studies have mentioned that important streamflow reduction and changes in flow regimen produce significant impacts on the abundance, composition, and diversity of aquatic communities (Brasher, 2017; Rolls and Bond, 2017).

Flow velocity averaged  $0.20 \text{ m/s}$  for sites P1 to P3. However, at the remaining sites, it presented high variability from  $0.04$  to  $0.32 \text{ m/s}$  (Table 1). In general, channel modifications are the main causes that explain the variability of flow velocity (Gregory, 2006). For instance, in the MRB, we observed an improvised weir that generated a pond near-site P4, a high level of dredging at P5, and a large channel width reduction at P6. These impacts together with the high levels of deforestation and erosion observed in the middle and lower reaches (P3 to P6) influenced various physicochemical parameters, such as water

**Table 1**  
Streamflow and levels of riverine condition along the MRB. Condition is reciprocal to the average level of impact of selected indicators according to Celi et al. (2018).

Indicator	Sites					
	P1	P2	P3	P4	P5	P6
<i>Streamflow</i>						
Discharge (m <sup>3</sup> /s)	0.055	0.062	0.043	0.024	0.020	0.018
Flow velocity (m/s)	0.19	0.21	0.18	0.04	0.12	0.32
<i>Riverine condition</i>						
Deforestation	Low	Medium	High	High	High	High
Erosion	Low	Medium	High	High	High	High
Dredging level	Absent	Low	Medium	Medium	High	Medium
Water extraction or weirs	Low	Low	Medium	High	Medium	Medium
Channel width variation	Low	Low	Medium	Medium	High	High

temperature, conductivity, dissolved solids, ions (see sections 3.2 and 3.3), aggravating the impacts on aquatic communities (Damnik-Ambarita et al., 2016b).

3.2. Physicochemical and microbiological characterization

Physicochemical and microbiological outcomes (Fig. 2; Table S3) suggested the downstream degradation of water quality along the MRB due to deforestation, erosion, and channel modification derived from agriculture and livestock. Various parameters related to the aforementioned activities (i.e., COD, conductivity, TDS, and total/fecal coliforms) exceeded maximum permissible levels for both Ecuadorian and American water quality regulations.

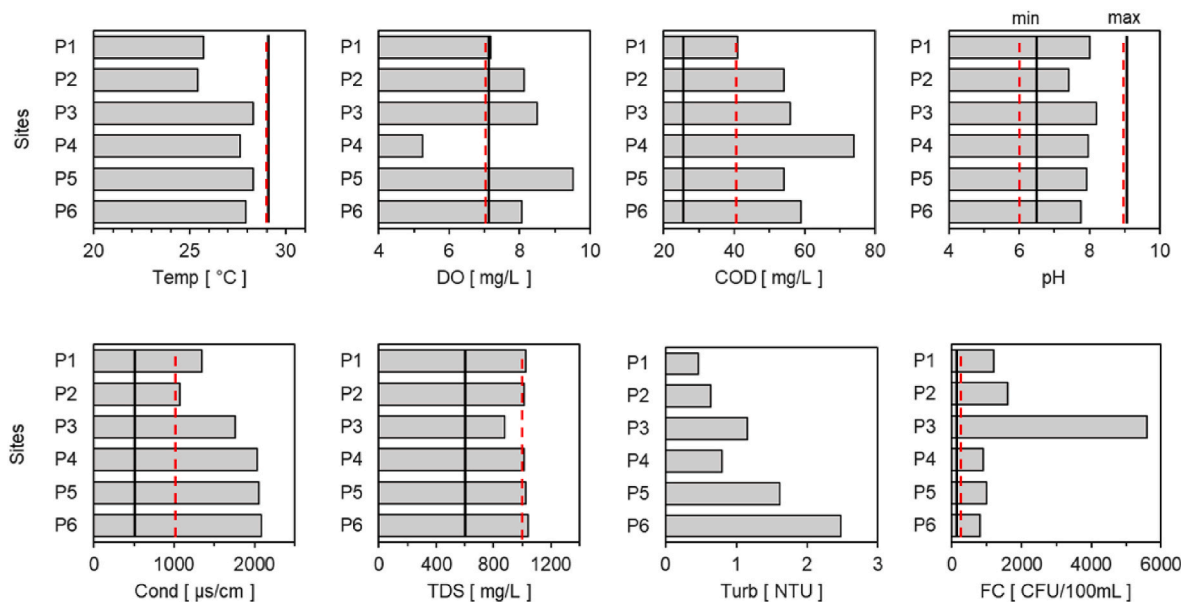
At the headwaters, water temperature averaged 25.5 °C, while in the middle and lower reaches it averaged 28 °C. This behavior is explained by the forest distribution since vegetation cover modifies the energy balance on river water by intercepting solar radiation (Lozano-Parra et al., 2018). Sites with the highest deforestation levels along the MRB were those with the highest water temperatures. On the other hand, dissolved oxygen (DO) levels complied with the requirements established in water quality regulations (Fig. 2; Table S3), except at site P4 where DO was 5.2 mg/L. Chemical oxygen demand (COD), a parameter that influences the oxygenation conditions of water (Palma et al., 2018), increased downstream along the MRB. Note that site P4 presented the maximum COD level (74 mg/L) and the lowest DO level. This is

explained by the significant reduction in flow velocity (Table 1) which produces a strong accumulation of organic matter derived from the intense livestock activities observed upstream.

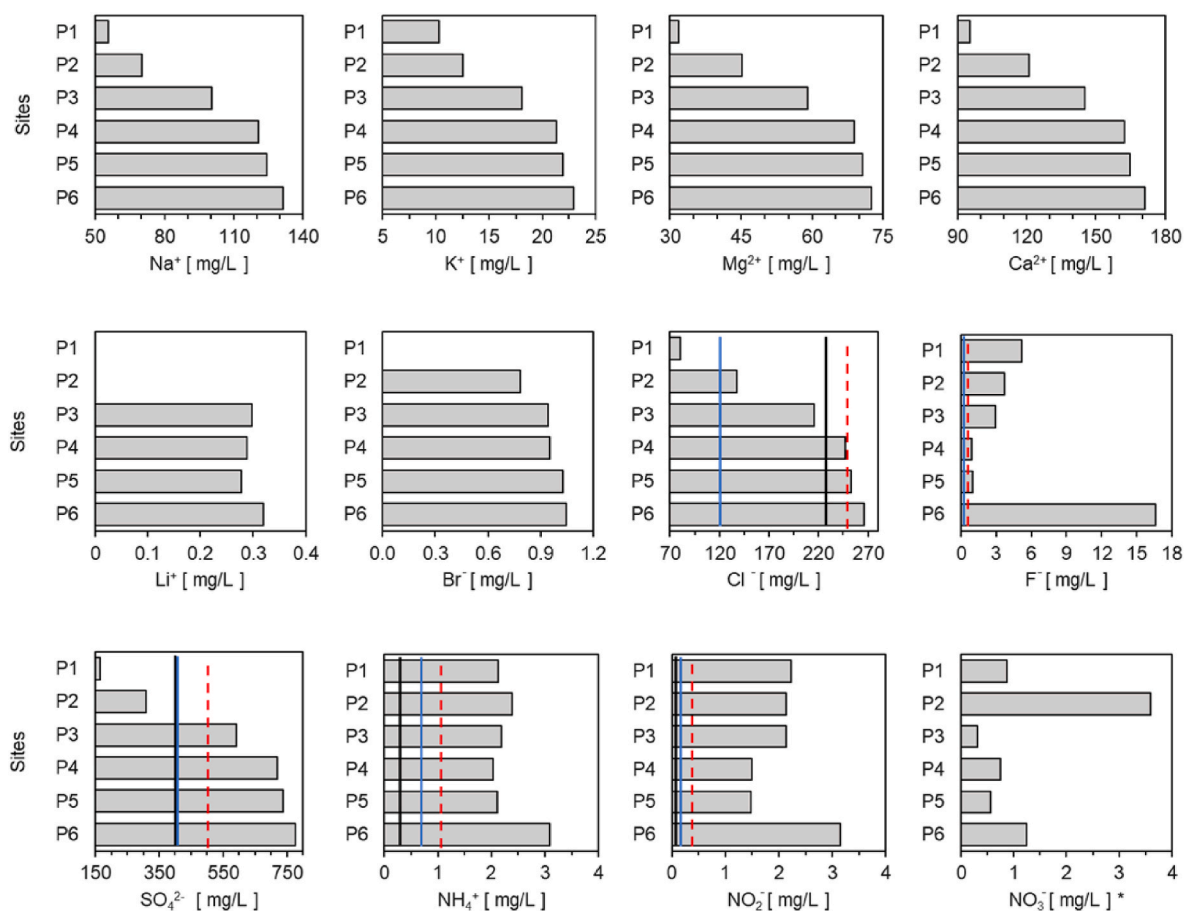
Overall, pH varied between 7.7 and 8.2, values that are within the limits established by American and Ecuadorian water quality regulations (Fig. 2). Conductivity ranged from 1072 to 2409 us/cm and total dissolved solids (TDS) presented values between 880 and 1205 mg/L (Table S3). Turbidity showed a high increase from headwaters to lower reaches (0.28–2.48 mg/L). These results are typical of basins with high levels of agriculture and livestock near river banks as the case of the MRB, since these activities stimulate erosion and sediment transport along the basin (Kosmowska et al., 2016; Margenat et al., 2017; Ríos-Villamizar et al., 2017). Finally, microbiological parameters showed levels above the established threshold in Ecuadorian and American water quality regulation (Fig. 2; Table S3). Site P3 presented alarming results, as it reached 56,000 CFU/100 mL and 324,000 CFU/100 mL for fecal and total coliforms, respectively (Table S3). In this site, a high density of cattle was observed living on the river banks.

3.3. Ion composition

Ion concentrations gradually increased from the headwaters to lower reaches, especially major cations and anions (Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup>, SO<sub>4</sub><sup>-</sup>, Fig. 3). Similar trends have been reported in basins that present deforestation, agriculture, and animal husbandry along their river banks



**Fig. 2.** Physicochemical and microbiological parameters measured along the MRB: water temperature (Temp), dissolved oxygen (DO), chemical oxygen demand (COD), pH, conductivity (Cond), total dissolved solids (TDS), turbidity (Turb), and fecal coliforms (FC). Water quality criteria for the protection of aquatic life and irrigation: red dashed lines for Ecuadorian regulation (MAE, 2015) and black lines for American regulation (USEPA, 2017, 1986). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 3.** Concentration of analyzed ions: Lithium ( $\text{Li}^+$ ), Sodium ( $\text{Na}^+$ ), Potassium ( $\text{K}^+$ ), Magnesium ( $\text{Mg}^{2+}$ ), Calcium ( $\text{Ca}^{2+}$ ), Fluorides ( $\text{F}^-$ ), Chlorides ( $\text{Cl}^-$ ), Bromide ( $\text{Br}^-$ ), Phosphate ( $\text{PO}_4^{3-}$ ), Sulphates ( $\text{SO}_4^{2-}$ ), Ammonia ( $\text{NH}_4^+$ ), Nitrites ( $\text{NO}_2^-$ ), Nitrates ( $\text{NO}_3^-$ ). Water quality criteria for the protection of aquatic life and irrigation presented by Ecuadorian (red dashed lines; MAE, 2015), American (black lines; USEPA, 1986, 2017) USEPA, and Canadian (blue lines; CCME, 2002, 2008) regulations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Margenat et al., 2017; Potaszniak and Szymczyk, 2015).

No lithium was detected at headwater, but its concentrations averaged 0.29 mg/L in the middle and lower reaches (Fig. 3). We discard that  $\text{Li}^+$  release occurs naturally in the MRB as regional geology does not present significant pegmatite contents, the main natural source of  $\text{Li}^+$  in the environment (Burbano et al., 2006; Kavanagh et al., 2017). Similarly, no bromide was found at site P1 but it varied between 0.7 and 1.2 mg/L in the remaining sites. Chloride concentrations ranged from 80 to 337 mg/L and all sites, except P1, exceeded the maximum threshold presented in Canadian regulation. Fluoride decreased from P1 to P5, however, an excessive  $\text{F}^-$  concentration (16.6 mg/L) was found at site P6. Note that all sites presented  $\text{F}^-$  concentrations above permissible limits described in regulations (Fig. 3; Table S3). Various authors such as Flury and Papritz (1993) and Négrel et al. (2010) describe that enrichment of these ions ( $\text{Li}^+$ ,  $\text{Br}^-$ ,  $\text{Cl}^-$ ,  $\text{F}^-$ ) is produced by diffuse contamination of agriculture as several agrochemicals contain them.

Regarding nitrogenous ions, concentrations of ammonia and nitrite varied from 2.0 to 3.1 mg/L and 1.4–3.1 mg/L, respectively. These values were 5 to 10-fold higher than maximum thresholds established by regulations (Fig. 3; Table S3). In contrast, nitrate concentrations ranged between 0.31 and 1.24 mg/L, except at site P2 where it was 3.88 mg/L. No site reached the maximum  $\text{NO}_3^-$  threshold concentrations. Overall, high concentrations of  $\text{NH}_4^+/\text{NO}_2^-$  respect to  $\text{NO}_3^-$  at DO levels (5.2–9.0 mg/L) found in the MRB suggest recent pollution by nitrogen fertilizer (e.g., urea) and livestock wastewater discharge (Lehtovirta-Morley, 2018; Xia et al., 2018).

### 3.4. Organophosphate pesticides (OPPs)

From seven OPPs analyzed in this study, Dimethoate, Sulfotep, Parathion, and Malathion were detected along the MRB. At headwaters, malathion showed high concentrations, exceeding thirteen-fold the maximum threshold established by the American regulation. Malathion exceeded the threshold in all sites, even reaching 27.8  $\mu\text{g/L}$  at site P6 (Table 3). These values were above concentrations detected in other river basins of western Ecuador, such as the Guayas river basin where Malathion ranged from 0.022 to 0.687  $\mu\text{g/L}$  and was mainly related to rice fields, maize, sugarcane, and cacao plantations (Deknock et al., 2019). We expected high Malathion levels as it is the most widely used OPP in Ecuador due to its multiple applications in agriculture, livestock, pet care, and vector-borne disease control (Hoffman et al., 2000; Mateo-Sagasta and Burke, 2011). Malathion is considered to have low toxicity for humans, but it is very toxic for fishes and microcrustaceans, causing endocrine-disrupting and reproductive effects (Brun et al., 2005; Fadaei et al., 2012).

Dimethoate was detected at site P6 in a concentration below the threshold established in the regulations (Table 3). This OPP is very toxic to aquatic organisms and moderately toxic for humans (Agrawal et al., 2010). Note that dimethoate is a degradable chemical, highly soluble in water and highly mobile in soil, and could infiltrate into the groundwater rapidly until it gets undetectable on the surface water (Köck-Schulmeyer et al., 2014; Lorenzo-Flores et al., 2017). This generates concern about groundwater that is used as a secondary source of water by the inhabitants due to the water deficit.

Sulfotep concentrations (0.011–0.022 µg/L) were lower compared to the rest of analyzed OPPs (Table 3). Sulfotep is used as a fumigant in greenhouses and can be found in nature as an aerosol. This makes it easier to spread by the air than through surface water or even less through groundwater (Chbib et al., 2018). Several studies suggest that the presence of low sulfotep concentrations, as the case of the MRB, is due to the fact that this pesticide can be found as an impurity in other agrochemicals such as Diazinon and Chlorpyrifos (Hala et al., 2016; Mekonen et al., 2016; Mojiri et al., 2020).

Although Parathion has been banned in Ecuador since 2009 (Agrawal et al., 2010; Agrocalidad, 2019), it has been detected in higher concentrations (0.028–0.346 µg/L) than the average reported in other surface waters (0.001–0.150 µg/L) of countries where its use is totally prohibited (Gao et al., 2009; Hoffman et al., 2000; Na et al., 2006). Note that parathion exceeded 9-folds the American regulation (Table 3). These results raise concern since this OPP is highly toxic and extremely hazardous for humans and can cause irreversible damage to fish and aquatic macroinvertebrate communities (Rico et al., 2010).

### 3.5. Macroinvertebrate composition and diversity

Aquatic macroinvertebrates found along the MRB corresponded to 17 families (10 orders), and totaled 506 individuals (Table S4). Pollution-sensitive (PS) families that are considered indicators of good

water quality represented only 8.7 % of the total macroinvertebrate abundance (Dominguez-Granda et al., 2011; Everaert et al., 2014; Sundar et al., 2020). In contrast, the three most abundant families were pollution-tolerant (PT): Thiaridae with 272 individuals (54 % of the total abundance), followed by Elmidae and Pachychillidae with 97 (19 %) and 53 (10 %) individuals, respectively (Fig. 4a).

These three aforementioned PT families have been widely reported in river water with high conductivity and ion concentrations (Beermann et al., 2018; Nguyen et al., 2017; Olson and Hawkins, 2017; Zhao et al., 2016). Similar results were reported in other high agricultural and livestock impacted river basins of western Ecuador, such as Portoviejo River Basin (Van Echelpoel et al., 2018) and Guayas River Basin (Damanik-Ambarita et al., 2016b). In the MRB, abundance of PT individuals increased from P1 to P5 as downstream ion concentrations and deforestation levels increased (Table S4; Fig. 4b). However, at P6 a significant decrease in abundance was observed. High pesticide concentrations in aquatic ecosystems induce a decrease in the abundance of macroinvertebrates (Palma et al., 2018; Schäfer, 2019). In fact, maximum pesticide concentrations were found at P6, which may justify the decrease in abundance.

It is known that high diversity values ( $H' \rightarrow 5$ ;  $L \rightarrow 1$ ) are mainly related to pH, high flow velocity, high DO levels, and low TDS concentrations (Damanik-Ambarita et al., 2016b; Palma et al., 2018). In the present study, site P3 showed the greatest diversity (Fig. 4c) and the

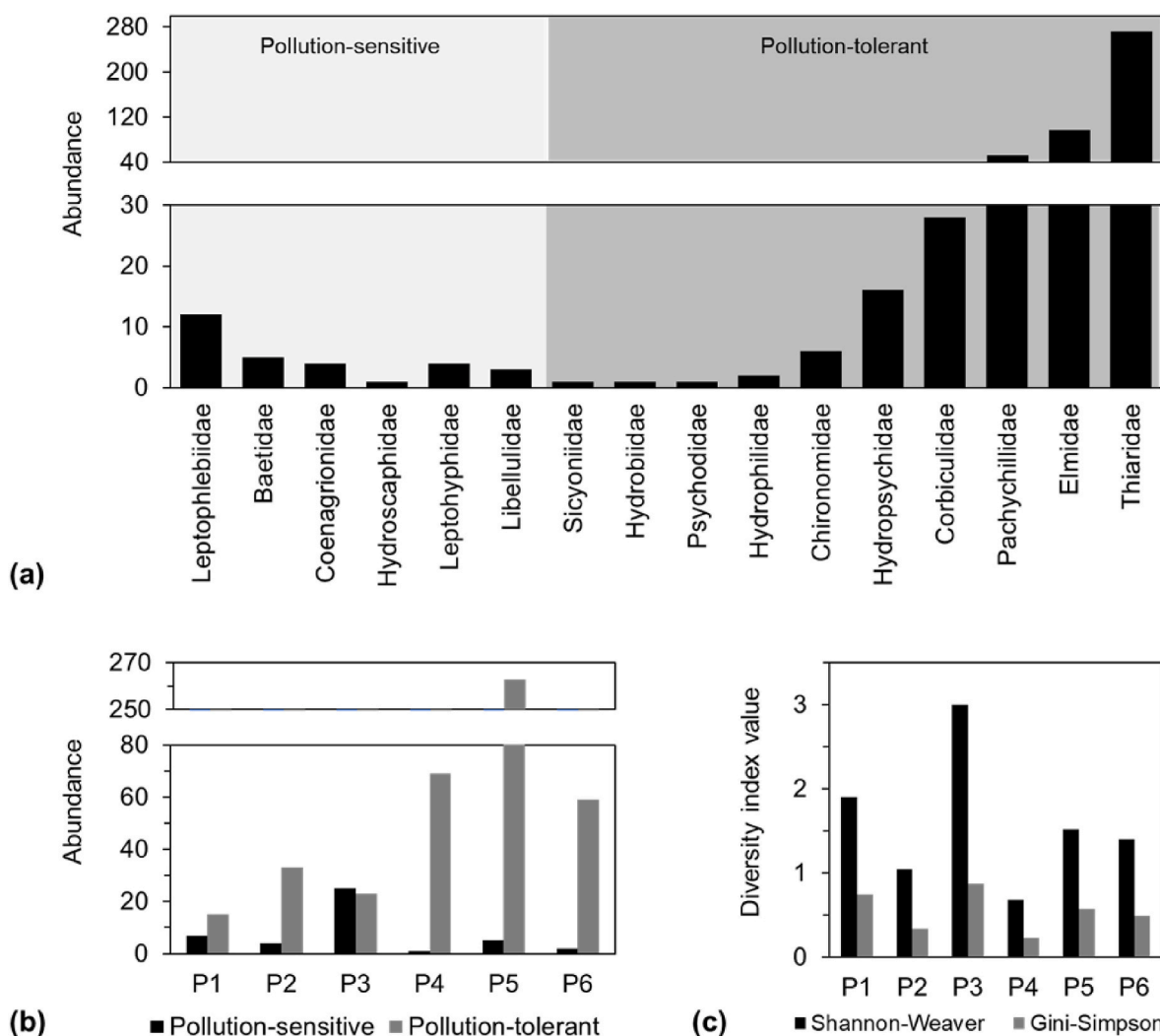


Fig. 4. Characterization of the macroinvertebrate community along the MRB. (a) Total abundance of macroinvertebrates families considering the whole MRB. (b) Abundance of PS and PT families per sampling site. (c) Diversity indices per sampling site.



lowest TDS concentration (880 mg/L). Instead, site P4 showed poor diversity and the lowest values for both flow velocity (0.04 m/s) and DO level (5.24 mg/L). It is interesting that P3 presented the highest diversity, being a site with the second highest malathion concentration (Table 2). Maltby and Hills (2008) mentioned that in cases in which no evident effects are observed in macroinvertebrates communities despite the strong pesticide pollution, the effects are evident at the individual level in their behavior or enzymatic activity. This type of analysis was not carried out in our study, but we agree with other authors that these analyzes should be included in the evaluation of aquatic ecosystems (Kwok et al., 2007; Maltby and Hills, 2008; Rico et al., 2011).

### 3.6. Phytotoxicity test

Phytotoxicity tests using *Lactuca sativa* showed low germination percentages in sediment (25–83%) with respect to water (73–93%). Seedlings showed growth inhibition in water samples ( $p < 0.05$ ) and growth enhancement in sediment ( $p < 0.05$ ; Fig. 5). Overall, low germination and growth inhibition represent signals of toxicity and they are influenced by pH, elevated concentrations of metals and ions (Capparelli et al., 2020; Chan-Keb et al., 2018; Galarza et al., 2021). Growth enhancement, otherwise, may indicate accumulation of organic matter and nutrient excess, and hence eutrophication (Capparelli et al., 2020; Galarza et al., 2021).

In water samples, pH values ranged from 7.7 to 8.2, discarding effects of acidity but suggesting the influence of ionic strength and toxic/trace elements (metals) concentrations in water toxicity (Biruk et al., 2017; Capparelli et al., 2020). In fact, several studies (e.g., Bauer-Gottwein et al., 2008; Biczak et al., 2017; Rout and Shaw, 2001; Simons, 2012; Tavakkoli et al., 2011; Young et al., 2016) describe that increase in dissolved ions on freshwater, especially  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$ , as the case of the MRB, affects the development of root tissues and membranes of plants (phytotoxicity) due to the change in ionic balance. On the other hand, various authors have mentioned that phytotoxicity would be more related to the concentration and bioavailability of metals (Amari et al., 2017; Capparelli et al., 2020; Margenat et al., 2017), however, in this study no metal concentrations were analyzed.

In sediment samples, the seedling growth enhancement was related to a strong accumulation of nutrients in sediments since high concentrations of nitrate (0.3–3.9 mg/L) and phosphate (1.2–1.7 mg/L) were reported in water samples of the MRB (Table S3). Nevertheless, low germination indicates that not only nutrients are being concentrated in sediments, but also a complex mixture of pollutants (Galarza et al., 2021). Note that the lowest germination percentage corresponded to sites P6 and P3, where the maximum pesticide concentrations in water were reported (Table 3). Finally, an interesting finding is the notable increase in growth enhancement, which indicates that in the sediment matrix the cumulative effect of agricultural and livestock activities is

**Table 2**

Sulfotep, Dimethoate, Malathion and Parathion concentrations in water collected along the MRB. Results were compared with maximum permissible limits from TULSMA (MAE, 2015), USEPA (USEPA, 1986, 2017), and CCME (CCME, 2002, 2008).

Station	Pesticide concentration (ug/L)			
	Sulfotep	Dimethoate	Malathion	Parathion
P1	N/D	N/D	1.245	N/D
P2	N/D	N/D	1.462	N/D
P3	0.013	N/D	<b>4.288</b>	0.031
P4	0.011	N/D	0.250	0.028
P5	0.019	N/D	1.491	0.034
P6	0.022	0.031	<b>27.856</b>	<b>0.346</b>
TULSMA	Total sum of organophosphate pesticides < 10 µg/L			
USEPA	–	0.50	0.10	0.04
CCME	–	0.62	–	–

N/D: No detected. Pesticide concentration below detection limit, 0.010 µg/L.

**Table 3**

Integration of multiple lines of evidence (LOEs) to assess the freshwater ecosystem health along the MRB. HMC: hydromorphological condition index. NSF-WQI: National Sanitation Foundation Water Quality Index. BMWP: Biological Monitoring Working Party. ASPT: Average Score Per Taxon. EPT: Ephemeroptera Plecoptera and Trichoptera. GI: germination index based on phytotoxicity tests with *L. sativa* for water and sediment. IFEQ: Integrative Freshwater Ecosystem Quality. All values are normalized to 0–100 scale.

Line of evidence/Index	Sites					
	P1	P2	P3	P4	P5	P6
<i>Hydromorphological LOE</i>						
HMC	73.3	53.3	26.7	20.0	8.7	13.3
<i>Physicochemical LOE</i>						
NSF-WQI	51.5	50.7	41.1	44.2	48.9	48.3
CCME-WQI	30.6	27.9	26.3	29.7	29.6	25.0
<i>Biological LOE</i>						
BMWP	16.7	29.3	50.0	15.8	39.2	25.0
ASPT	50.0	34.0	58.0	49.0	48.0	45.0
EPT	18.2	10.8	47.9	0.0	1.5	3.3
<i>Phytotoxicological LOE</i>						
GIwater	37.3	53.3	45.7	43.8	37.1	41.9
GI sediment	53.9	51.0	32.9	73.7	57.4	29.1
<i>Integrative LOEs</i>						
IFEQ	44.4	40.5	36.8	31.1	26.3	25.5

more evident than in water.

### 3.7. Integrative analysis and final remarks

In general, each LOE can characterize different pollution sources and drivers of aquatic ecosystem degradation (Galarza et al., 2021), however, the integrative framework that uses IFEQ index also captured the synergistic of degradation processes produced by human activities and their cumulative effects, confirming the high decrease of freshwater ecosystem health along the MRB (Table 3).

The hydromorphological (LOE 1), physicochemical (LOE 2), and phytotoxicological (LOE 4) indices presented moderately good values at headwaters where native forest is the matrix on the landscape (Table 3). This suggests that headwaters present relatively better ecosystem health compared to middle and lower reaches (P3 to P6). As we expected, the multivariate analysis (PCA and HCA) grouped sites P1 and P2 such as the less threatened groups (Fig. 6). However, headwaters presented important pollution by Malathion and the biological water quality indices (LOE 3) showed disappointing results. The IFEQ index reached 44.4 and 40.5 for sites P1 and P2, respectively, which indicates poor freshwater ecosystem conditions. These results agree with the reality of the region since headwater degradation of agricultural basins of western Ecuador has been widely reported in previous studies (Damaniik-Ambarita et al., 2018; Nguyen et al., 2017; Parker and Carr, 1992; Van Echelpoel et al., 2018).

Despite site P3 reached the maximum values of biological water quality indices along the MRB (~50, Table 3), results still suggested a poor water quality. In fact, the IFEQ index was 36.8, confirming a bad freshwater ecosystem condition. Note that hydromorphological, physicochemical, and phytotoxicological indices showed a notable reduction at site P3 compared to headwater due to deforestation, streamflow reduction, and enrichment of ions and pesticides. However, the effects of these four aforementioned degradation drivers are more evident at sites P4, P5, and P6 (Fig. 6). Overall, sites P4 to P6 showed the lowest scores for all LOEs, confirming that intense agricultural and livestock activities represented significant impacts on the aquatic ecosystem of the MRB, such as other authors previously discussed for basins with similar threats (Morabowen et al., 2019). The IFEQ classified site P4 with a bad condition (31.2), whereas sites P5 and P6 with critical conditions (<30).

As we expected, the multivariate analysis separated site P6 from other sites of middle and lower reaches due to its high concentration of Malathion, Parathion, and Dimethoate (Fig. 6). The observed critical

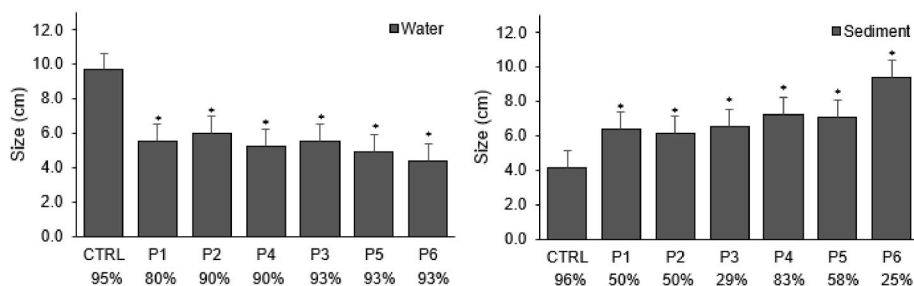


Fig. 5. Responses of *Lactuca sativa* to water and sediment phytotoxicity assays. Phytotoxicity was evaluated by comparing the plant size (root + hypocotyl) exposed to water and sediments taken from sampling sites compared to the lab control (CTRL) using Wilcoxon test. Data means ± standard deviation, and \* indicates a statistical difference ( $p < 0.05$ ). The percentages values below site names indicate the seed germination.

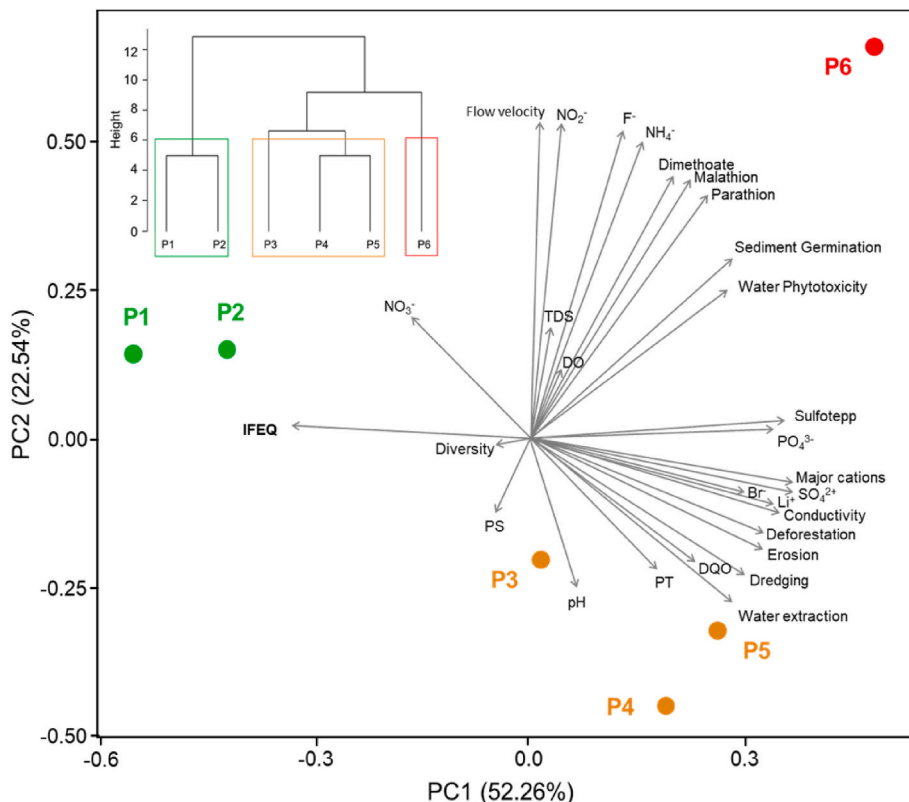


Fig. 6. Relation among sampling sites and hydromorphological, physicochemical, biological, and phytotoxicological variables obtained in the MRB based on the Principal Component Analysis (main figure) and Hierarchical Cluster Analysis (inside figure). Sites P1 and P2 are located at the headwaters whereas sites P3 to P6 correspond to middle and lower reaches of the MRB

OPP pollution in the whole MRB constitutes a potential risk to human health. For that reason, we agree with other authors that the use of highly toxic pesticides within basins and near rivers should be strictly regulated (Chaikasem and Na Roi-Et, 2020). Furthermore, training workshops are essential due to the main reason for OPP pollution is the lack of knowledge of farmers and inhabitants about necessary dosage, methods, regulations, and suitable time of pesticide application (Fadaei et al., 2012).

The lack of effective public policies for headwaters conservation and regulation of intense agricultural and livestock activities that result in pesticide and ion release has played an important role in freshwater ecosystems degradation of western Ecuador. Thus, this integrative framework that combines multiple LOEs and better explains the cumulative effects of human impacts, should be replicated in basins with similar situations in order for decision-makers and concerned inhabitants to generate adequate policies and strategies to mitigate the degradation of freshwater ecosystems.

### Author contributions

**Juseth E. Chancay:** Writing- Original draft, Conceptualization, Formal analysis, Data Curation, Visualization, Investigation. **Oscar Lucas-Solis:** Conceptualization, Writing- Original draft, Formal analysis, Visualization. **Daniela Alvear-S:** Data Curation, Writing- Original draft, Methodology, Investigation. **Dayana Martínez-R:** Investigation, Data Curation, Writing- Original draft. **Gisella Mena:** Investigation, Methodology, Writing- Original draft. **Bryan Zurita:** Investigation, Writing- Original draft. **Luis Carrasco-S:** Investigation, Writing- Original draft. **Henry Joel Carrillo:** Investigation, Writing- Original draft. **Víctor Segarra:** Investigation, Writing- Original draft. **Elizabeth Nar-anjo:** Investigation, Writing- Original draft. **Brian Coronel:** Investigation, Writing- Original draft. **Rodrigo Espinosa:** Conceptualization, Writing - Review & Editing. **Marcela Cabrera:** Validation, Writing - Review & Editing. **Mariana V. Capparelli:** Conceptualization, Investigation, Writing - Review & Editing, Supervision. **Jorge E. Celi:**

Conceptualization, Writing - Review & Editing, Project administration, Supervision.

### Author statement

This manuscript is not under consideration for publication elsewhere and authors have no competing interests to declare.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2021.117796>.

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