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Bryophyte Communities along a Tropical Urban River Respond to Heavy Metal and Arsenic Pollution

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Abstract: Aquatic and rheophilous bryophytes can indicate water pollution as they bioaccumulate toxic water elements. We evaluated (1) bioaccumulation of eight heavy metals and arsenic by *Marchantia polymorpha* L., and (2) changes in bryophyte community structure, as responses to urban pollution in southern Ecuador. To this end, we registered presence/absence and coverage of submerged bryophytes in 120 quadrats across three zones of the Zamora river inside Loja city, and a control zone in a nearby forest. We found that the concentrations of five (Al, Cd, Cu, Fe, and Zn) of the eight chemical elements and arsenic were highest in urban *M. polymorpha*. Moreover, bryophyte species richness decreased in urban zones. Bryophyte community structure also differed between control and city zones, but no differences were found among city zones. The control zone was composed by a more distinct set of bryophyte species, e.g., an indicator species analysis showed that 16 species had high and significant indicator values for control zone, but only 11 species were indicators of at least one of the three urban zones. We concluded that bryophytes, in general, and *M. polymorpha*, in particular, can be suitable biomonitors of water quality in tropical urban rivers.

Keywords: community structure; diversity; indicator species; *Marchantia polymorpha* L.; passive monitoring

1. Introduction

Communities occurring along urban rivers are increasingly near sources of pollution such as municipal wastewater, domestic garbage, and agricultural and industrial discharges [1–3]. Most of these effluents contain toxic substances, like heavy metals and metalloids, which are a considerable threat to the environment [4,5], and human health [6–9]. As such, looking for a sensitive and effective indicator of water pollution is an important task for scientists and local authorities that can inform decision making and city planning [10].

Bryophytes are well-known bioaccumulators of toxic elements due to its eco-physiology (i.e., rapid absorption and slow desorption of pollutants [10–12]), and morphology (bryophytes lack of epidermis that allows them to accumulate toxins present in water [13,14]). Recent studies have shown that some bryophyte species living in contaminated rivers, like the thallose liverwort *Marchantia polymorpha* L., can be enriched by heavy metals like Cu, Zn, and Cd [12,13]. Moreover, bryophyte community structure can also respond to natural and anthropogenic variability throughout a river's profile. For instance, it has been shown that bryophyte communities change with turbidity, water temperature, and pH in the Italian Alps and Apennines [15,16]. Water pollution [10] and level of urbanization [15,17–20] also have an impact in other European bryophyte community structures.

Despite their great importance as bioindicators and bioaccumulators, most bryophyte studies have been carried out in temperate regions of the world [10]. In Ecuador, most bryophyte studies have been taxonomic in scope [21–24] and few studies has focused on monitoring water contamination using aquatic macroinvertebrate communities [25]. Here, we present the first analysis of the effects of water pollution on bryophyte bioaccumulation of eight heavy metals and one metalloid. We further test if bryophyte community structure, beyond effects on individual species, responds to river pollution. To this end, we measured bryophyte species richness and community composition in the Zamora river, passing through Loja city, a major city in southern Ecuador. We asked the following questions: (1) Is *M. polymorpha* bioaccumulating toxic elements inside Loja city? (2) How do bryophyte species richness and community structure respond to water pollution? (3) Are there bryophyte species strictly associated either to forest or city areas? Previous research has shown a higher concentration of pollutants in centric zones of cities [10,14,20,26–29].

2. Materials and Methods

2.1. Study Area

We performed this study in the streams and river banks of the Zamora river, passing through Loja city; which has been eroded by wastewater contamination, garbage, and extraction of stony material [30]. The study area corresponds to urban zones and a forest fragment of Loja city. The approximate area of the city is 10,790 km^2. The annual average temperature of Loja is 15 °C, and the annual precipitation is about 900 mm [30]. The study consisted of three study zones (south, center, and north) within city limits and along the river, and a control zone (forest) outside the city. We sampled 3 sites within each zone, for a total of 12 sites (Figure 1).

The control zone (F) includes the upper parts of the river basin, with banks dominated by forest remnants [25]. The south zone (S; 1035.000 m^2 of area or recreational parks; 68,919 inhabitants), is characterized by recent urban development and lack of adequate supply of sewage. According to the water quality index Canadian Council Ministers (CCME-WQI) and water quality index (WQI-C), the water is considered to be regularly contaminated or poor in quality. Arsenic, mercury, and lead in the rivers reach values up to 0.326 ppm, 0.022 ppm, and 0.389 ppm, respectively [31]. The center zone (C; 635,000 m^2 of area; 54,576 inhabitants) is characterized by a high degree of urbanization and high number of effluents of sewage. According to the CCME-WQI and the WQI-C, the water is considered to be highly contaminated or poor in quality. In this area, levels of arsenic, mercury, and lead can reach values up to 0.043 ppm, 0.175 ppm, and 0.664 ppm, respectively [31]. The north zone (N; 1060.000 m^2 of area; 26,527 inhabitants), finally, is an urban area with a high storage of microbiological load due to sewage [30]; nevertheless, the zone still has some recreational parks. According to the CCME-WQI and WQI-C, the water is considered to be highly contaminated or poor in quality. The levels of arsenic, mercury, and lead in this zone can reach values up to 0.022 ppm, 0.043 ppm, and 0.688 ppm, respectively [31].

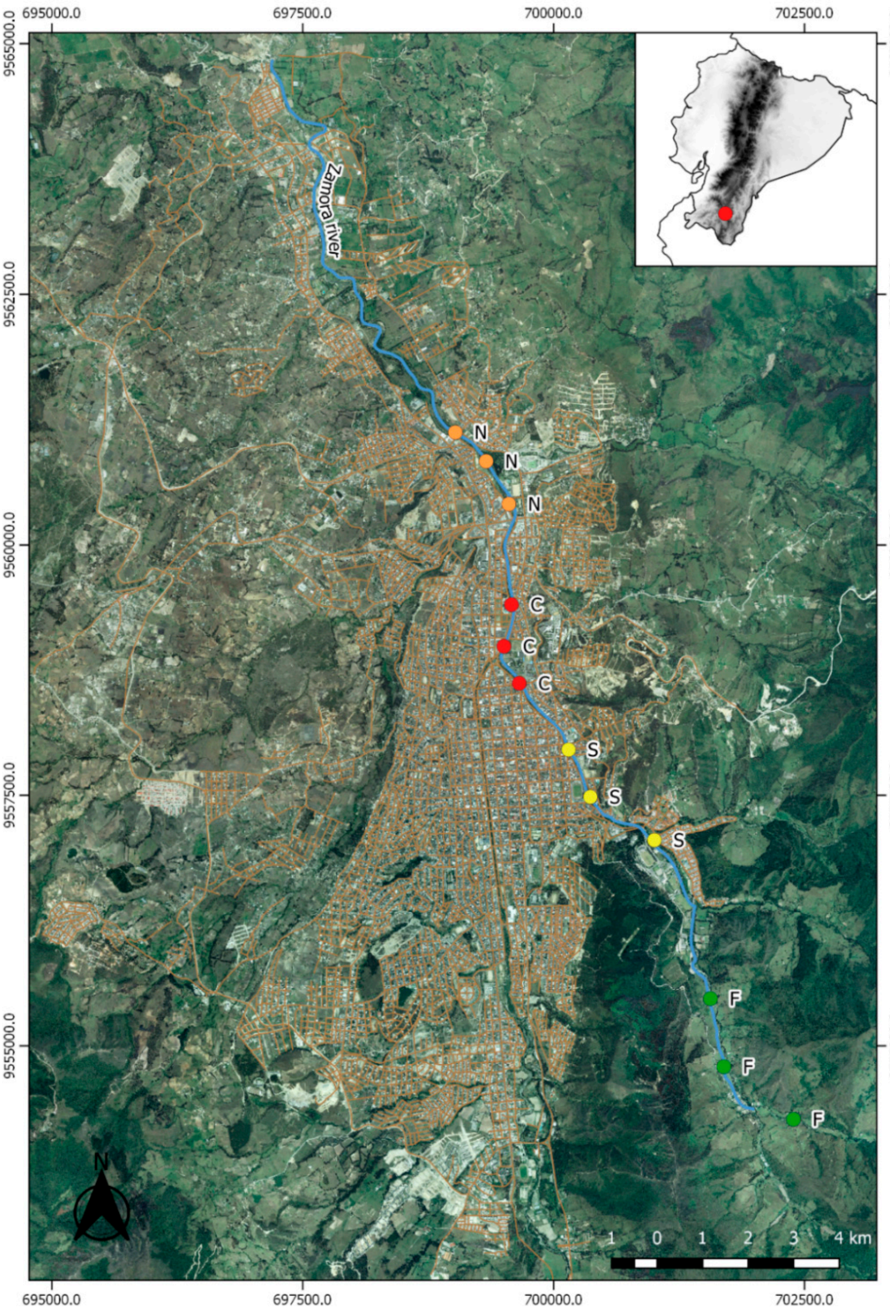

Figure 1. Study area in Loja Province in southern Ecuador showing the location of four study zones. Control zone (F), south zone (S), center zone (C), and north zone (N).

2.2. *Elemental Bioccumulation in Marchantia polymorpha* L.

Elemental bioaccumulation was studied in the subcosmopolitic thallose liverwort *Marchantia polymorpha* L. We chose this species because it has proved elsewhere to be an effective bioaccumulator of heavy metals [12,13,32]. To sample *M. polymorpha*, we took four samples (0.5–1 g) of the species in the same sites (two separate sites by zone), where bryophytes frequency and cover were sampled at each of the four study zones. They were rinsed with Milli-Q water, then stored in paper bags for oven drying at 60 °C for 3–4 days. Fieldwork was conducted between April and December 2015.

The dried samples were mechanically milled using a stainless-steel grinder for digestion. Subsequently, 0.2 g of dry sample was weighed, then a preparation of aqua regia (HCl and HNO_3) was performed in a 3:1 ratio (v/v) [33]. Each sample was digested twice with 30 ml of aqua regia subjected to a heating plate with a temperature of 250 °C. After the samples were allowed to cool, the solutions were filtered through filter paper in a 100 ml distillation flask calibrated with distilled water. The concentrations of eight heavy metals—aluminum (Al), cadmium (Cd), copper (Cu), iron (Fe), magnesium (Mn), mercury (Hg), lead (Pb), and zinc (Zn), and one metalloid, arsenic (As)—in digested solutions were analyzed using a flame atomic absorption spectrophotometer Perkin Elmer AAnalyst 400 (Shelton, CT, USA). The respective wavelength (nm), precision (as the relative standard deviation, %), and limit of detection (μg g^{-1}) of the elements were as follows: Pb 283.31, 1.0 and, 0.05; Cu 324.75, 1.2, and 0.010; Cd 228.80, 1.7, and 0.002, Mg 285.21; Zn 213.86, 2.31, and 0.005; Fe 305.91, 0.01; Al 309.27, 0.1. A witness was taken into account to estimate metal contamination in the digestion process. All elements used here are certified, and were acquired from AccuStandard, Inc. (125 Market Street New Haven, CT 06513, USA), a company accredited to ISO Guide 34, ISO/IEC 17025, and certified to ISO 9001.

2.3. Bryophyte Community Structure

In each of the 12 zones, ten 20 × 30 cm quadrats were selected along the banks of the Zamora river [34]. In each quadrat, we registered presence/absence and coverage of rheophilous (permanently submerged) and aquatic (periodically submerged) bryophytes. The samples were identified using general [35,36] and specific keys [37] and were deposited as vouchers in the herbarium HUTPL at Universidad Técnica Particular de Loja.

2.4. Data Analysis

To determine changes in heavy metal accumulation of *M. polymorpha* across the different zones, we used one-way analysis of variance (ANOVA) followed by a Tukey post-hoc test. Shapiro Wilk and Bartlett's test confirmed the model met normality and homogeneity of variance assumptions. However, when data were non-normal, we used Kruskal–Wallis one-way ANOVA on ranks followed by Dunn's pairwise comparison. Analyses were done in R 3.2.2 [38] using the "dunn.test" package [39].

We calculated species richness as the total number of different bryophytes species occurring in a quadrat. We also calculated sampling completeness with the *Chao 2* species richness estimator using the R package 'vegan' [40]. The effect of environmental variables (zone, site, slope, and plant cover) on species richness was modeled by fitting generalized linear mixed models (GLMMs) [41] as implemented in the R package 'nlme' [42]. The minimal adequate model was selected based on Akaike's information criterion (AIC). Data were analyzed from a multi-level approach, considering site as a random factor and introducing the environmental variables as fixed factors (zone, plant cover, and slope). To identify significant richness differences between zone, a post hoc Tukey multiple comparison tests as implemented in the R package 'lsmeans' [43]. The richness models of bryophytes were fitted with Poisson errors.

To detect for changes in bryophyte community structure among zones we used a permutational multivariate analysis of variance (PERMANOVA) on the species cover matrix [44]. Experimental design included two factors: Zone (four levels, fixed factor), site (three levels, random factor nested within zone), plant cover (fixed factor), and slope (fixed factor), with 10 replicate quadrats for each locality (120 quadrats). We used the Bray–Curtis distance measure and 999 Monte Carlo permutations. We visualized community level composition with a non-metric multidimensional scaling (NMDS) on the species cover matrix. NMDS and PERMANOVA were conducted in vegan.

To determine which bryophyte species was associated with specific zone, we used the indicator species analysis [45] as implemented in the R package 'labdsv' [46]. This analysis calculates an indicator value for each species based on the mean cover of each species per zone, which results from multiplying the relative abundance for each species by the frequency for each species in each zone. The indicator value ranges from 0 to 1, or 0 to 100. The significance was tested using a Monte Carlo permutation with 1000 replicates.

3. Results

3.1. Elemental Bioaccumulation by Marchantia Polymorpha

The concentrations of heavy metals (Cd, Cu, Mn, Pb, Zn, Al, and Fe) and the metalloid arsenic in *M. polymorpha* significantly differed in the four zones (Figure 2). A greater concentration of Al, Cu, Fe, Mn, and Zn in *M. polymorpha* was measured in the three urbanized zones when compared with the control (Table 1 and Figure 2). No traces of Hg were detected across the sites, thus we removed Hg from further analysis.

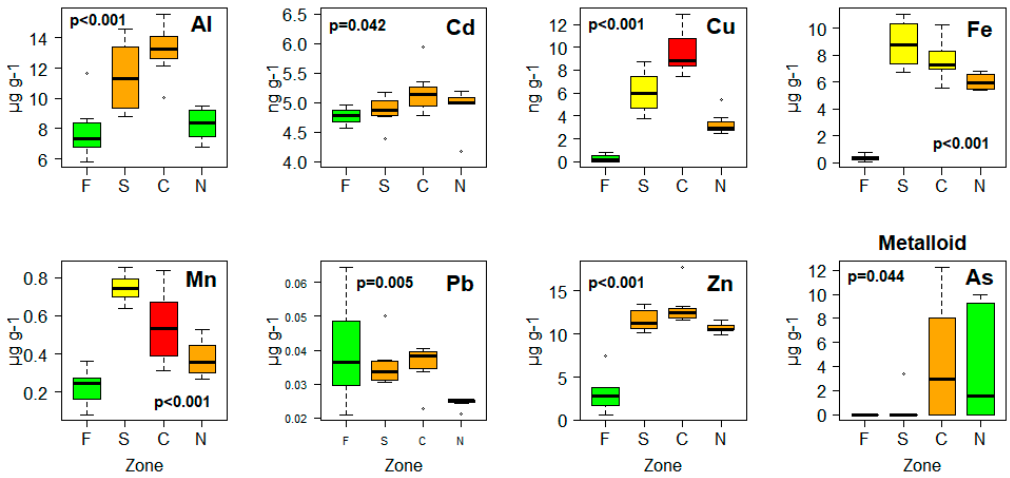

Figure 2. Boxplot of heavy metals and metalloid accumulation in the *Marchantia polymorpha* collected from different zones in the Zamora River. Al, Cd, Fe, Mn, and Zn: One-way ANOVA followed by a Tukey test. As, Cu, and Pb: Kruskal–Wallis ANOVA followed by a Dunn's test. Different colors for each zone are considered significant ($p < 0.05$).

Table 1. Mean concentration (µg g^{-1}) and standard error (SE) of heavy metals and arsenic in *Marchantia polymorpha* by different zones.

		Control		South		Center		North	
Element		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Heavy Metals	Al	7.82	1.7	11.43	2.1	13.21	1.5	8.31	0.9
	Cd	0.01	0	0.01	0	0.01	0	0.01	0
	Cu	0	0	0.01	0	0.01	0	0	0
	Fe	0.34	0.2	8.84	1.5	7.61	1.3	6.03	0.6
	Hg	0	0	0	0	0	0	0	0
	Mn	0.23	0.1	0.75	0.1	0.54	0	0.37	0.1
	Pb	0.04	0	0.04	0	0.04	0	0.03	0
	Zn	3.08	2	11.64	1.2	13.02	1.9	10.71	0.5
Metalloid	As	0	0	0.42	1.1	4.28	4.7	3.94	4.4

3.2. Species Richness

A total of 44 bryophyte species were recorded, including 24 mosses, 18 liverworts, and 2 anthocerotes. The control zone showed the highest richness with 34 species, followed by the south zone (29 spp.), the central zone (24 spp.), and the north zone (21 spp.) (Figure 3). A similar pattern was observed with the *Chao 2* richness estimator, confirming a high number of species estimated in the control zone (41 estimated species), followed by the south, center, and north zones (with 33, 26, and 22 estimated species, respectively).

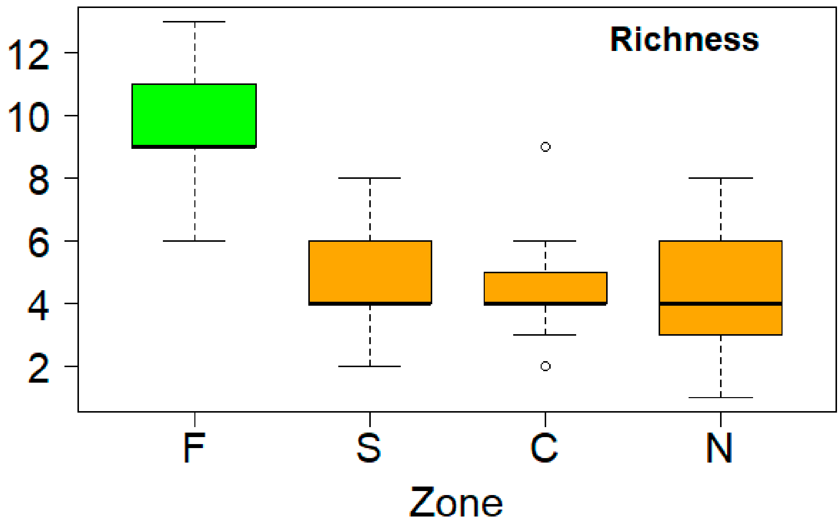

Figure 3. Boxplot of bryophyte species richness at different zones along the Zamora river. Different colors correspond to post hoc Tukey groups ($p < 0.05$) after a GLMM. Control zone (F); south zone (S); center zone (C); and north zone (N).

Results of the mixed models showed that the most relevant predictor for the bryophytes richness was the control zone (Table 2).

Table 2. Results of the generalized linear mixed models (GLMM) on bryophytes richness and environmental variables (zone, site, slope, and plant cover). The random variable site was non-significant.

Factor	Estimate	Z-value	p
Control	0.754	7.038	<0.001
South	0.103	0.858	0.391
Center	0.026	6.772	0.172
North	0.05	0.391	0.696
Slope	0.001	0.862	0.389
Plant cover	−0.002	−0.569	0.569

3.3. Bryophyte Community Structure

PERMANOVA showed that bryophyte communities varied significantly both at the zone ($R^2 = 0.22$; $p = 0.001$) and at the site ($R^2 = 0.16$; $p = 0.001$) but not plant cover ($R^2 = 0.01$; $p = 0.096$) (Table 3). Slope, while significant ($p = 0.01$), explained only 1% of the variance.

Table 3. Results of PERMANOVA analysis of species composition and environmental variables (zone, site, slope and plant cover). $p < 0.05$ are considered significant. Df = degrees of freedom; SS = sum of squares; MS = Mean squares; F = statistical; R^2 = coefficient of variation.

Source	Df	SS	MS	F	R^2 (CV)	p
Zone	3	9.16	3.05	12.50	0.22	0.001
Site	8	6.78	0.85	3.47	0.16	0.001
Plant cover	1	0.39	0.40	1.61	0.01	0.096
Slope	1	0.52	0.52	2.14	0.01	0.019
Residuals	103	25.15	0.24		0.60	
Total	116	42.00			1	

The NMDS plot (Stress = 0.20) showed that species composition of the Control zone (Forests) was significantly different from that of the other three zones (Figure 4).

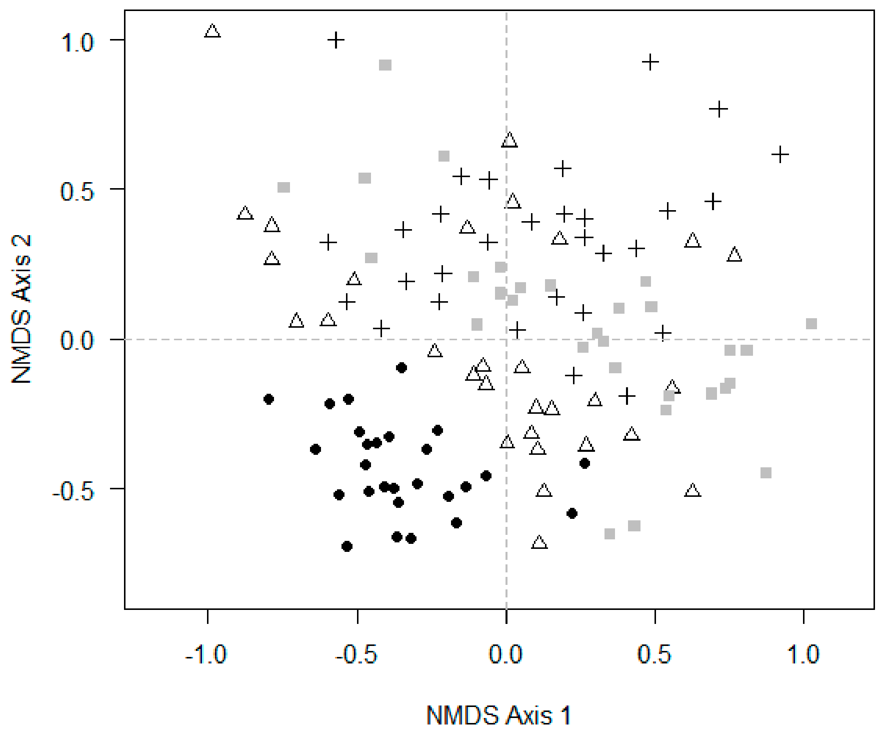

Figure 4. Non-metric multidimensional scaling (NMDS) ordination plot for the samples (quadrats) for Control (●), South (Δ), Center (+), and North (■).

3.4. Indicator Species Analysis

We determined 16 indicator species of the control zone, 3 indicator species in the south, and 4 indicator species both in the north and central zones (Table 3). *Fissidens serratus* Müll. Hal and *Monoclea gottschei* Lindb., were the best indicator of Control zone. *Noteroclada confluens* (Hook.f. and Taylor) Spruce showed high indicator value for South zone. *Campylopus pauper* (Hampe) Mitt. and *Marchantia polymorpha* L. showed high indicator value for Center zone and *Fissidens elegans* Brid., for the North zone (Table A1).

4. Discussion

We found higher concentrations of four heavy metals (Al, Cu, Fe, and Zn) in *M. polymorpha* in city zones, which indicated its ability to bioaccumulate toxic elements. Thus, this species can be used as an effective biomonitor of heavy metal pollution [12]. Our study adds *M. polymorpha* to the list of suitable species for this task. For example, several studies have highlighted the ability of *Fontinalis antipyretica* to absorb heavy metals (i.e., Al, Cu, Cr, Cd, and Zn) and As in rivers with urban, industrial, and agricultural residual discharges [14,27,47]. Other bryophyte species used for this purpose in temperate regions include: *Platyhypnidium riparioides*, *Fissidens polyphyllus*, *Brachythecium rivulare*, *Hygrohypnum ochraceum*, *Fontinalis antipyretica*, *F. squamosa*, *F. dalecarlica*, *F. duriaei*, *Thamnobryum alopecurum*, and *Taxiphyllum barbieri* [10,27,28,48–50]. Our study, however, comes with a caveat; *M. polymorpha* is sometimes exposed to air, thus it can bioaccumulate air pollution in addition to water pollution. Future research should evaluate both the average time *M. polymorpha* remains submerged in a year and *M. polymorpha's* abilities to accumulate air contamination.

As expected, bryophyte species richness was highest in the Control (forest) zone. Ceschin et al. [51] have showed that greater bryophyte richness occurs in fast-flowing rivers, clean waters with high oxygenation, and good habitat characteristics for the growth of bryophytes (e.g., temperature, pH, turbidity). However, low bryophyte richness in urban zones of the Loja river might be explained in part by the absence of natural vegetation in the banks of the river, which results in less available habitat for the growth of bryophytes, and in part to the presence in the riverbed of metal walls and wooden covers [52]. For example, Downes et al. [53] found that urban parts of rivers affect negatively bryophyte establishment and distribution.

Bryophyte community structure also changed significantly between control and urban zones, but no differences as one enters to the city zones. These differences were attributed to a greater frequency and coverage of sensitive species restricted to zones of natural forests where less urbanization and water pollution allow them to grow more. Thus, Scarlett and O'Hare [17] and Ceschin et al. [51] pointed that most sensitive bryophyte prefer clear, oxygenated waters with low concentrations of ammonium and phosphates. In our case, bryophytes communities in the control zone were better represented by species such as *Symphyogyna brongniartii*, *Rhodobryum huillense*, *Plagiochila laetevirens*, *Monoclea gottschei*, *Dumortiera hirsuta*, *Neesioscyphus argillaceus*, and *Fissidens serratus* (Appendix A), which have been previously identified as sensitive to changes in water quality [54–56].

Nonetheless, tolerant species were able to grow in waters of the center and north zones of the river, which present high levels of contamination, lack of oxygen, and greater amount of organic waste. For instance, *Platyhypnidium aquaticum*, *M. polymorpha*, *Rhynchostegium scariosum*, *Thuidium delicatulum*, and *Riccia crassifrons* are typical of disturbed habitats [51,55–57], which agrees with our results (Appendix A). In addition, some studies point that *Platyhypnidium aquaticum* and *Brachythecium rivulare* are characteristic species of open environment with poor water conditions caused by urban spills [11,51,58].

The indicator species analysis showed that *Clasmatocolea vermicularis*, *Fissidens serratus*, *Lophocolea bidentata*, *Monoclea gottschei*, *Noteroclada confluens*, *Symphyogyna brongniartii*, *Symphyogyna brasiliensis*, *Plagiochila laetevirens*, and *Rhodobryum huillense* were the best indicators of the control zone, related to uncontaminated water. Accordingly, *Lophocolea bidentata*, *Symphyogyna brongniartii*, and *Monoclea gottschei* occur more abundantly in shady habitats with vegetation of undisturbed forests, due to the fact that they are sensitive to environmental changes [59,60]. Conversely, *Fissidens elegans*, *Riccia crassifrons*, and *Marchantia polymorpha* were the best indicators of more urbanized zones such as in southern, central, and northern areas with greater impact of anthropogenic disturbances. Some authors point out that these species are considered tolerant to pollution, and therefore can be indicative of intense urban activity related with wastewater [19].

Our results revealed significant changes in bryophyte community structure, reduced species richness, and increases in heavy metal concentration in *M. polymorpha* in the Zamora river as you move inside Loja City. These results are likely a consequence of a greater degree of urbanization and wastewater deposition in the river and its tributaries. We conclude that bryophyte species richness decreases in urban zones; similarly, species composition changes significantly in the control zone with respect to urban zones of the river. These changes likely reflect higher concentration of heavy metals (Al, Cd, Cu, Fe, and Zn) in urban zones, which likely reflect the impact of human settlements and wastewater related water pollution. The complementary use of the diversity and bioaccumulation of heavy metals in bryophytes can provide key information of water pollution of Zamora River of Ecuador, which, in the long term, will allow for the establishment of monitoring zones for adequate management and strategies of mitigation of water pollution. Although our study demonstrates the efficacy of bryophyte in passive biomonitoring, we suggest that future work should evaluate bryophyte as part of active biomonitoring where transplantation of bryophytes allows for the evaluation and monitoring of the magnitude of water pollution due to the known exposure period [10,61].

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Indicator values (IV) for bryophyte species. Species with indicator value >25 are considered as the best indicators. Species with both high indicator value and a significant *p* value ($p < 0.05$) are shown in bold.

Species	Zone	IV	*p*
Anthocerophyta			
Anthoceros punctatus L.	Control	3.7	0.23
Phaeoceros laevis (L.) Prosk.	South	15.7	0.11
Marchantiophyta			
Clasmatocolea vermicularis (Lehm.) Grolle	South	12.4	0.01
Dumortiera hirsuta (Sw.) Nees	Control	**33.4**	**<0.01**
Lejeunea cerina Lehm. & Lindenb.	Control	**33.3**	**<0.01**
Lophocolea bidentata (L.) Dumort.	Control	**48.1**	**<0.01**
Lophocolea connata (Sw.) Nees	South	9.7	0.06
Lophocolea sp.	Center	19.6	0.02
Marchantia chenopoda L.	North	**22.1**	**0.01**
Marchantia polymorpha L.	Center	**31.4**	**<0.01**
Monoclea gottschei Lindb.	Control	**69.0**	**<0.01**
Neesioscyphus argillaceus (Nees) Grolle	Control	**25.8**	**<0.01**
Neesioscyphus sp.	South	14.6	0.02
Noteroclada confluens (Hook.f. & Taylor) Spruce	South	**28.4**	**<0.01**
Plagiochila laetevirens Lindenb.	Control	**40.7**	**<0.01**
Riccardia regnellii (Ångstr.) G.K.Hell	Control	17.5	0.01
Riccia crassifrons Spruce	North	23.2	0.01
Symphyogyna brasiliensis Nees	Control	**40.7**	**<0.01**
Symphyogyna brongniartii Mont.	Control	**55.3**	**<0.01**
Bryophyta			
Brachythecium aff. *serrulatum* (Hedw.) H. Rob.	Control	7.0	0.11
Campylopus sp. 1	South	2.7	0.11
Campylopus sp. 2	South	4.4	0.65
Campylopus pauper (Hampe) Mitt.	Center	**27.7**	**<0.01**
Didymodon tophaceus (Brid.) Lisa	Control	8.3	0.11
Fissidens elegans Brid.	North	**33.3**	**<0.01**
Fissidens serratus Müll. Hal.	Control	**62.6**	**<0.01**
Fissidens weirii Mitt.	Center	13.1	0.16
Hookeria acutifolia Hook. & Grev.	Control	7.4	0.05
Philonotis sp. 1	Center	3.0	0.84
Philonotis sp. 2	South	5.6	0.28
Philonotis sp. 3	Control	14.8	0.03
Philonotis sp. 4	Control	7.4	0.04
Plagiomnium medium (Bruch & Schimp.) T. Kop.	Control	4.7	0.27
Platyhypnidium aquaticum (A. Jaeger) M. Fleisch.	South	15.3	0.21
Pseudocrossidium sp.	North	23.3	<0.01
Rhodobryum beyrichianum (Hornsch.) Müll. Hal.	North	8.8	0.23
Rhodobryum huillense (Welw. & Duby) Touw	Control	**27.9**	**<0.01**
Rhodobryum procerum (Schimp.) Paris	Center	9.5	0.08
Rhynchostegium riparioides (Hedw.) Cardot	South	4.2	0.34
Rhynchostegium scariosum (Taylor) A. Jaeger	Control	3.3	0.58
Sematophyllum sp.	North	12.7	0.42
Sematophyllum subsimplex (Hedw.) Mitt.	North	10.0	0.53
Thuidium delicatulum (Hedw.) Mitt.	Control	**30.4**	**<0.01**
Thuidium sp.	Control	23.2	<0.01

References

1. Postel, S.; Richter, B. Rivers for life: Managing water for people and nature. Potovelo-Zaruma, Ecuador. *J. Clean. Prod.* **2003**, *18*, 226–232.
2. Groffman, P.M.; Bain, D.J.; Band, L.E.; Belt, K.T.; Brush, G.S.; Grove, J.M.; Pouyat, R.V.; Yesilonis, I.C.; Zipperer, W.C. Down by the riverside: Urban riparian ecology. *Front. Ecol. Environ.* **2003**, *1*, 315–321. [CrossRef]
3. Richardson, D.M.; Holmes, P.M.; Esler, K.J.; Galatowitsch, S.M.; Stromberg, J.C.; Kirkman, S.P.; Pyšek, P.; Hobbs, R.J. Riparian vegetation: Degradation, alien plant invasions, and restoration prospects. *Divers. Distrib.* **2007**, *13*, 126–139. [CrossRef]
4. Karadede, H.; Unlu, E. Concentrations of some heavy metals in water, sediment and fish species from the Atatürk Dam Lake (Euphrates), Turkey. *Chemosphere* **2000**, *41*, 1371–1376. [CrossRef]
5. Igwe, J.C.; Abia, A.A. Maize Cob and Husk as Adsorbents for removal of Cd, Pb and Zn ions from wastewater. *Phys. Sci.* **2003**, *2*, 83–94.
6. Miller, C.V.; Foster, G.D.; Majedi, B.F. Baseflow and stormflow metal fluxes from two small agricultural catchments in the coastal plain of Chesapeake Bay Basin, United States. *Appl. Geochem.* **2003**, *18*, 483–501. [CrossRef]
7. Boularbah, A.; Schwartz, C.; Bitton, G.; Aboudrar, W.; Ouhammou, J.L. Heavy metal contamination from mining sites in South Morocco: 2. Assessment of metal accumulation and toxicity in plants. *Chemosphere* **2006**, *63*, 811–817. [CrossRef]
8. Schwarzenbach, R.; Egli, T.; Hofstetter, T.; Gunten, U.; Wehrli, B. Global Water Pollution and Human Health. *Annu. Rev. Environ. Resour.* **2010**, *35*, 109–136. [CrossRef]
9. Ali, H.; Khan, E.; Sajad, M.A. Phytoremediation of heavy metals concepts and applications. *Chemosphere* **2013**, *91*, 869–881. [CrossRef] [PubMed]
10. Debén, S.; Aboal, J.; Carballeira, A.; Cesa, M.; Real, C.; Fernandez, J. Inland water quality monitoring with native bryophytes: A methodological review. *Ecol. Indic.* **2015**, *53*, 115–124. [CrossRef]
11. Vanderpoorten, A. Aquatic bryophytes for a spatio-temporal monitoring of the water pollution of the rivers Meuse and Sambre (Belgium). *Environ. Pollut.* **1999**, *104*, 401–410. [CrossRef]
12. Ares, Á.; Itouga, M.; Kato, Y.; Sakakibara, H. Differential Metal Tolerance and Accumulation Patterns of Cd, Cu, Pb and Zn in the Liverwort *Marchantia polymorpha* L. *Bull. Environ. Contam. Toxicol.* **2018**, *100*, 444–450. [CrossRef]
13. Sharma, S. *Marchantia polymorpha* L.: A Bioaccumulator. *Aerobiologia* **2007**, *23*, 181–187. [CrossRef]
14. Tipping, E.; Vincent, C.D.; Lawlor, A.J.; Lofts, S. Metal accumulation by stream bryophytes, related to chemical speciation. *Environ. Pollut.* **2008**, *156*, 936–943. [CrossRef]
15. Ceschin, S.; Bisceglie, S.; Aleffi, M. Contribution to the knowledge of the bryoflora in running waters of Central Italy. *Plant Biosyst.* **2012**, *146*, 622–627.
16. Ceschin, S.; Minciardi, M.R.; Spada, C.D.; Abati, S. Bryophytes of Alpine and Apennine mountain streams: Floristic features and ecological notes. *Cryptogam. Bryol.* **2015**, *36*, 267–283. [CrossRef]
17. Scarlett, P.; O'Hare, M. Community structure of in-stream bryophytes in English and Weslsh rivers. *Hydrobiologia* **2006**, *553*, 143–152. [CrossRef]
18. Nimis, P.L.; Fumangalli, F.; Bizzotto, A.; Codogno, M.; Skert, N. Bryophytes as indicators of trace metals pollution in the River Brenta (NE Italy). *Sci. Total Environ.* **2002**, *286*, 233–242. [CrossRef]
19. Gecheva, G.; Pall, K.; Hristeva, Y. Bryophyte communities' responses to environmental factors in highly seasonal rivers. *Bot. Let.* **2017**, *164*, 79–91. [CrossRef]
20. Shevock, J.R.; Ma, W.-Z.; Akiyama, H. Diversity of the rheophytic condition in bryophytes: Field observations from multiple continents. *Bryophyt. Divers. Evol.* **2017**, *39*, 75–93. [CrossRef]
21. Benítez, A.; Prieto, M.; Aragón, G. Large trees and dense canopies: Key factors for maintaining high epiphytic diversity on trunk bases (bryophytes and lichens) in tropical montane forests. *Forestry* **2015**, *88*, 521–527. [CrossRef]
22. Gradstein, S.R.; Benitez, A. Liverworts New to Ecuador with Description of *Plagiochila priceana* sp. nov. and *Syzygiella burghardtii* sp. nov. *Cryptogam. Bryol.* **2017**, *38*, 335–349. [CrossRef]
23. Castillo-Monroy, A.P.; Benítez, Á.; Reyes-Bueno, F.; Donoso, D.A.; Cueva, A. Biocrust structure responds to soil variables along a tropical scrubland elevation gradient. *J. Arid Environ.* **2016**, *124*, 31–38. [CrossRef]

24. Benitez, A.; Gradstein, S.R. Adiciones a la Flora de Briófitas del Ecuador. *Cryptogam. Bryol.* **2011**, *32*, 65–75. [CrossRef]
25. Iñiguez, C.; Leiva, A.; Georg, H.; Hampel, H.; Breuer, L. Deforestation and Benthic Indicators: How Much Vegetation Cover Is Needed to Sustain Healthy Andean Streams? *PLoS ONE* **2014**, *9*, 1–8.
26. Lopez, J.; Carballeira, A. Interspecific differences in metal bioaccumulation and plant-water concentration ratios in five aquatic bryophytes. *Hydrobiologia* **1993**, *263*, 95–107. [CrossRef]
27. Vuori, K.M.; Helisten, H. The use of aquatic mosses in assessment of metal pollution: Appraisal of type-specific background concentrations and inter-specific differences in metal accumulation. *Hydrobiologia* **2010**, *656*, 99–106. [CrossRef]
28. Gecheva, G.; Yurukova, L.; Ganeva, A. Assessment of Pollution with Aquatic Bryophytes in Maritsa River (Bulgaria). *Bull. Environ. Contam. Toxicol.* **2011**, *87*, 480–485. [CrossRef]
29. Gecheva, G.; Yurukova, L. Water pollutant monitoring with aquatic bryophytes: A review. *Environ. Chem Lett.* **2014**, *12*, 49–61. [CrossRef]
30. Programa de las Naciones Unidas para el Medio Ambiente. *Municipalidad de Loja & Naturaleza y Cultura Internacional, PNUMA*; Geo-Loja: Loja, Ecuador, 2007.
31. Maldonado, G. *Monitoreo de la calidad de agua del Río Malacatos, tramo comprendido desde los Dos Puentes hasta el sector de Sauces Norte*; Tesis pregrado, Universidad Técnica Particular de Loja: Loja, Ecuador, 2014.
32. Samecka-Cymerman, A.; Marczonek, A.; Kempers, A.J. Bioindication of heavy metals in soil by liverworts. *Arch. Environ. Contam. Toxicol.* **1997**, *33*, 162–171. [CrossRef]
33. Tack, F.; Verloo, M. Metal contents in stinging nettle (*Urtica dioica* L.) as affected by soil characteristics. *Sci. Total Environ.* **1996**, *192*, 31–39. [CrossRef]
34. Mandl, N.A.; Kessler, M.; Robbert Gradstein, S. Effects of environmental heterogeneity on species diversity and composition of terrestrial bryophyte assemblages in tropical montane forests of southern Ecuador. *Plant Ecol. Divers.* **2009**, *2*, 313–321. [CrossRef]
35. Gradstein, S.; Churchil, S.; Allen, N. Guide to Bryophytes of Tropical America. *N. Y. Bot. Gard.* **2001**, *88*, 5–570.
36. Churchill, S.P.; Linares, E.L. *Prodromus Bryologiae Novo-Granatensis. Part 1*; Instituto de Ciencias Naturales, Museo de Historia Natural, Universidad Nacional de Colombia: Bogotá, Colombia, 1995.
37. Gradstein, S.R.; Reeb, C. The Genus *Riccardia* (Aneuraceae) in Colombia and Ecuador. *Cryptogam. Bryol.* **2018**, *39*, 515–541. [CrossRef]
38. R Development Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2018.
39. Dinno A dunn.test: Dunn Test of Multiple Comparisons Using Rank Sums Package. R Package Version 1.3-5. 2017. Available online: https://cran.r-project.org/web/packages/dunn.test/ (accessed on 17 January 2019).
40. Oksanen, J.; Blanchet, F.G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; et al. Vegan: Community Ecology Package. R Package Version 2.5-4. 2019. Available online: https://cran.r-project.org/web/packages/vegan/ (accessed on 17 January 2019).
41. McCullagh, P. Some statistical properties of a new family of continuous univariate distributions. *J. Am. Statist. Assoc.* **1989**, *84*, 125–129. [CrossRef]
42. Pinheiro, J.; Bates, D.; DebRoy, S.; Sarkar, D. nlme: Linear and Nonlinear Mixed Effects Models Package. R Package Version 1.3-5. 2017. Available online: https://cran.r-project.org/web/packages/nlme/ (accessed on 17 January 2019).
43. Lenth, R.; Lenth, M.R. lsmeans: Least-Squares Means Package. R Package Version 2.30-0. 2018. Available online: https://cran.r-project.org/web/packages/lsmeans/ (accessed on 17 January 2019).
44. Anderson, M.J.; Gorley, R.; Clarke, K. *Permanova for PRIMER: Guide to Software and Statistical Methods*; PRIMER-E: Plymouth, UK, 2008.
45. Dufrêne, M.; Legendre, P. Species assemblages and indicator species: The need for a flexible asymmetrical approach. *Ecol. Monogr.* **1997**, *3*, 345–366. [CrossRef]
46. Roberts, D.W. labdsv: Ordination and Multivariate Analysis for Ecology Package. R Package Version 2.30-0. 2016. Available online: https://cran.r-project.org/web/packages/labdsv/ (accessed on 17 January 2019).
47. Davies, T.D. Sulphate toxicity to the aquatic moss, *Fontinalis antipyretica*. *Chemosphere* **2007**, *66*, 444–451. [CrossRef]

48. Martinez, J.; Garcia, A.; Beaucourt, N.; Nuñez, E. Combined seasonal and longitudinal variations of element concentrations in two aquatic mosses (*Fontinalis antipyretica* and *Fontinalis squamosa*). *Nova Hedwig.* **2002**, *74*, 349–364. [CrossRef]
49. Kováčik, J.; Babula, P.; Hedbavny, J. Comparison of vascular and non-vascular aquatic plant as indicators of cadmium toxicity. *Chemosphere* **2017**, *180*, 86–92. [CrossRef]
50. Favas, P.J.; Pratas, J.; Rodrigues, N.; D'Souza, R.; Varun, M.; Paul, M.S. Metal (loid) accumulation in aquatic plants of a mining area: Potential for water quality biomonitoring and biogeochemical prospecting. *Chemosphere* **2018**, *194*, 158–170. [CrossRef] [PubMed]
51. Ceschin, S.; Aleffi, S.; Savo, V.; Zuccarello, V. Aquatic bryophytes as ecological indicators of the water quality status in the Tiber River basin (Italy). *Ecol. Indic.* **2012**, *14*, 74–81. [CrossRef]
52. Francis, R.; Hoggart, S. Urban river wall habitat and vegetation: Observations from the River Thames through central London. *Urban Ecosyst.* **2009**, *12*, 465–485. [CrossRef]
53. Downes, B.; Entwisle, T.; Reicha, P. Effects of flow regulation on disturbance frequencies and in-channel bryophytes and macroalgae in some upland streams. *River Res. Appl.* **2003**, *19*, 27–42. [CrossRef]
54. Uribe Meléndez, J.; Aguirre Ceballos, J. Las especies colombianas del género *Symphyogyna* (hepaticae: Pallaviciniaceae). *Caldasia* **1995**, *17*, 82–85.
55. Linares, E.; Churchill, S. Comunidades de briófitos reofílicos en un Caño de montaña, en San Francisco, Cundinamarca, Colombia. *Caldasia* **1997**, *19*, 323–329.
56. Lagos, M.; Sáenz, F.; Morales, M. Briófitos Reófilos de tres Quebradas del páramo de Mamapacha, Chinavita (Boyacá-Colombia). *Acta Biol. Colomb.* **2008**, *13*, 143–160.
57. Belland, R.J.; Schofield, W.B. The ecology and phytogeography of the bryophytes of Cape Breton, Hyghlands National Park, Canada. *Nova Hedwig.* **1994**, *59*, 257–309.
58. Allegrini, M.C. The bryological flora and the chemical-physical characteristics of the water of the high course of the Sangro river (Abruzzo National Park). *Riv. Idrobiol.* **2000**, *39*, 9–20.
59. Ruiz, C.A.; Aguirre, J. Las comunidades de briófitos y su relación con la estructura de la vegetación fanerogamica, en el gradiente altitudinal de la Serranía del Perijá (Cesar–Colombia). *Bryophyt. Divers. Evol.* **2003**, *24*, 101–113.
60. Vargas, R.; Morales, E. Hepáticas del Parque Natural Municipal "Robledales de Tipacoque", Boyacá-Colombia. *Univ. Sci.* **2014**, *19*, 201–211. [CrossRef]
61. Debén, S.; Aboal, J.R.; Carballeira, A.; Cesa, M.; Fernández, J.A. Monitoring river water quality with transplanted bryophytes: A methodological review. *Ecol. Indic.* **2017**, *81*, 461–476. [CrossRef]