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The Use of Bryophytes, Lichens and Bromeliads for Evaluating Air and Water Pollution in an Andean City

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Abstract: Air and water pollution are global environmental problems; thus, bioindicators have become important tools for monitoring various pollutants, including metals and metalloids. *Parmotrema arnoldii* (Du Rietz) Hale and *Tillandsia usneoides* L. were evaluated as indicators of heavy metals in the air and *Platyhypnidium aquaticum* A. Jaeger and *Marchantia polymorpha* L. as indicators of heavy metals and a metalloid in water. The concentrations of cadmium (Cd), lead (Pb), copper (Cu), manganese (Mn) and zinc (Zn) as air pollutants and aluminum (Al), cadmium (Cd), iron (Fe), manganese (Mn), lead (Pb), zinc (Zn) and arsenic (As) as water pollutants were analyzed within four different zones (control, northern, central and southern) in an Andean city of Ecuador. The level of metal concentrations in the air for *P. arnoldii* and *T. usneoides* had the following order of concentration: Zn > Mn > Pb > Cd > Cu. In the case of water, *P. aquaticum* pointed out a concentration of Al > Mn > Fe > Zn > As > Pb > Cd and proved to be more effective in detecting water pollution than the species *M. polymorpha*, which had a concentration of Al >Zn > Fe > Cd >As > Mn > Pb. *P. aquaticum* showed a higher capacity to accumulate heavy metals than *M. polymorpha*; therefore, it can be used as a model species for passive water quality monitoring. However, *P. arnoldii* and *T. usneoides* showed similar heavy metal accumulation related to air quality. The passive monitoring of air quality using bromeliads and lichens as well as bryophytes for water quality proved their effectiveness and applicability in tropical regions such as Ecuador.

Keywords: passive biomonitoring; metal; lichen; bromeliad; mosses

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1. Introduction

The increase in the emission of toxic compounds in the recent years has been a growing problem, mainly affecting developing countries due to the growth of their population centers and energy and industrial activities [1,2]. As a result, air and water quality deteriorate severely [3–6]. The most frequent pollutants in cities are sulphur dioxide (SO_2), nitrogen oxides (NO_2) and heavy metals [7,8]. Every year, millions of people suffer from respiratory and other diseases caused by polluted air [9–11]. In addition, a similar number of people have gastrointestinal problems due to the use of contaminated water [12–14].

Bioindicators, for example, bromeliads [15–17], bryophytes [18–22] and lichens [5,23–25], are used to assess the quality of the environment and they are very effective in assessing air and water pollution by metals and other pollutants. These groups, due to their special morphological and ecophysiological structures [26–28], have the capacity to accumulate and retain pollutants, mainly due to their close relationship with the environment [2,29,30]. Therefore, their use has several advantages compared to traditional monitoring, where expensive chemical methods and equipment are used [31–33].

Biomonitors are a low-cost alternative for monitoring water and air quality [34–36]. Some of the most used are based on passive monitoring and focused on using native species of the different areas; for instance, several studies have focused on lichens [23,28,37,38], bryophytes [25,32,39,40] and vascular plants for determining air quality [30,41–44]. Aquatic bryophytes are also used to assess water quality [13,21,45–47]. However, few studies have incorporated different taxonomic groups for air and water quality monitoring; for instance, previous studies used a combination of lichens and bryophytes [25,28,48,49], lichens with vascular plants [50,51] and bryophytes with vascular plants for monitoring air pollution [40,42,52,53]. In terms of water pollution, studies of macroinvertebrates and bryophytes can be found [21,54,55]. However, in Ecuador, few studies have been carried out [24,47].

Air pollution in Ecuador is over 30%, more than the World Health Organization (WHO) recommended safe level, and is further aggravated by water pollution [56]. Therefore, alternatives are being sought to assess air and water quality for heavy metals. As a precedent, most studies have focused on using a single species for air quality monitoring [34,57] and water pollution [24,47]. Only one study has used lichens and bromeliads to determine air quality [50]; however, this study did not determine the accumulation effectiveness of the species.

We evaluated the effectiveness of bromeliads versus lichens as indicators of air quality, as well as the effectiveness of mosses versus liverworts as monitors of water pollution by heavy metals in the city of Loja, Ecuador. We hypothesized that that increased urbanization towards the geographic center of the city will result in increased bioaccumulation of heavy metals in epiphytic and aquatic species related to air and water pollution, respectively. This will allow us to determine model species for environmental pollution monitoring in the country, where studies related to air and water quality are limited. For example, in the large cities of Ecuador (Quito, Guayaquil and Cuenca), no studies have been performed to determine water quality and only one study in Quito has used a kind of vascular plant to identify air pollution [34].

2. Materials and Methods

2.1. Study Area

The study was conducted in urban areas and forested localities around the city of Loja, in the southern region of Ecuador at 2100 m.a.s.l. (Figure 1). The average annual temperature in the region is 20 °C, and it is characterized by an average relative humidity of about 80% [1]. For further information, see previous studies by Benítez et al. [50] related to air quality, and by Vásquez et al. [47] and Benítez et al. [24] focused on water quality in the city of Loja, Ecuador.

2.2. Design and Data Collection

We took five independent samples (0.5–1 g) of epiphyte species (*P. arnoldii* and *T. usneoides*) and aquatic species (*M. polymorpha and P. aquaticum*, Figure 2), at three independent sites within three urban zones (south, center, and north) and a forested zone (control) [24,47,50]. Control zone (F): The area is generally densely vegetated with a low human population and very little rural traffic. This zone includes the upper parts of the river basin, with banks dominated by fragments of evergreen tropical forest. This zone is characterized by a low level of water pollution [24,47,50]. Southern zone (S): This district is characterized by extensive green areas and recreational parks. This zone is subject to relatively high traffic due to the transit between this area and the city. The water is considered to be regularly contaminated or poor in quality, and is characterized by a high level of water pollution with metals such as aluminum, zinc, iron, manganese and metalloid arsenic [24,47,50]. Central zone (C): The downtown district is a mostly urban area with very little vegetation and high volumes of traffic. The water is considered to be contaminated or poor in quality with high levels of aluminum, zinc and iron [24,47,50]. Northern zone (N): This district has a relatively large quantity of green space and it is subject to moderate-high traffic. The water is considered to be contaminated or poor in quality with high levels of aluminum, zinc, iron, cadmium and sodium [24,47,50].

2.3. Chemical Analysis of Metals and Metalloid

Chemical analysis was performed using atomic absorption spectroscopy (AAnalyst 400; Perkin Elmer Sdn Bhd, Selangor, Malaysia), and the samples were sieved to remove the residues and dried in a drying oven at 50 °C. The digestion method requires the addition of 0.5 g of sample and 10 mL of HNO_3 in the digestion vessel [21]. After the digestion, the volume of each sample was adjusted to 100 mL using double deionized water. A microwave digestion system (MARS Microwave Accelerated Reaction System 6) by CEM Corporation was used. The concentration analyses of cadmium (Cd), copper (Cu), nickel (Ni), aluminum (Al), iron (Fe), manganese (Mn), lead (Pb) and zinc (Zn) were performed using calibration curves prepared with certified standards (MerckKGaA, Darmstadt, Germany) for each of the metals using curves generated with a high correlation coefficient (r > 0.995).

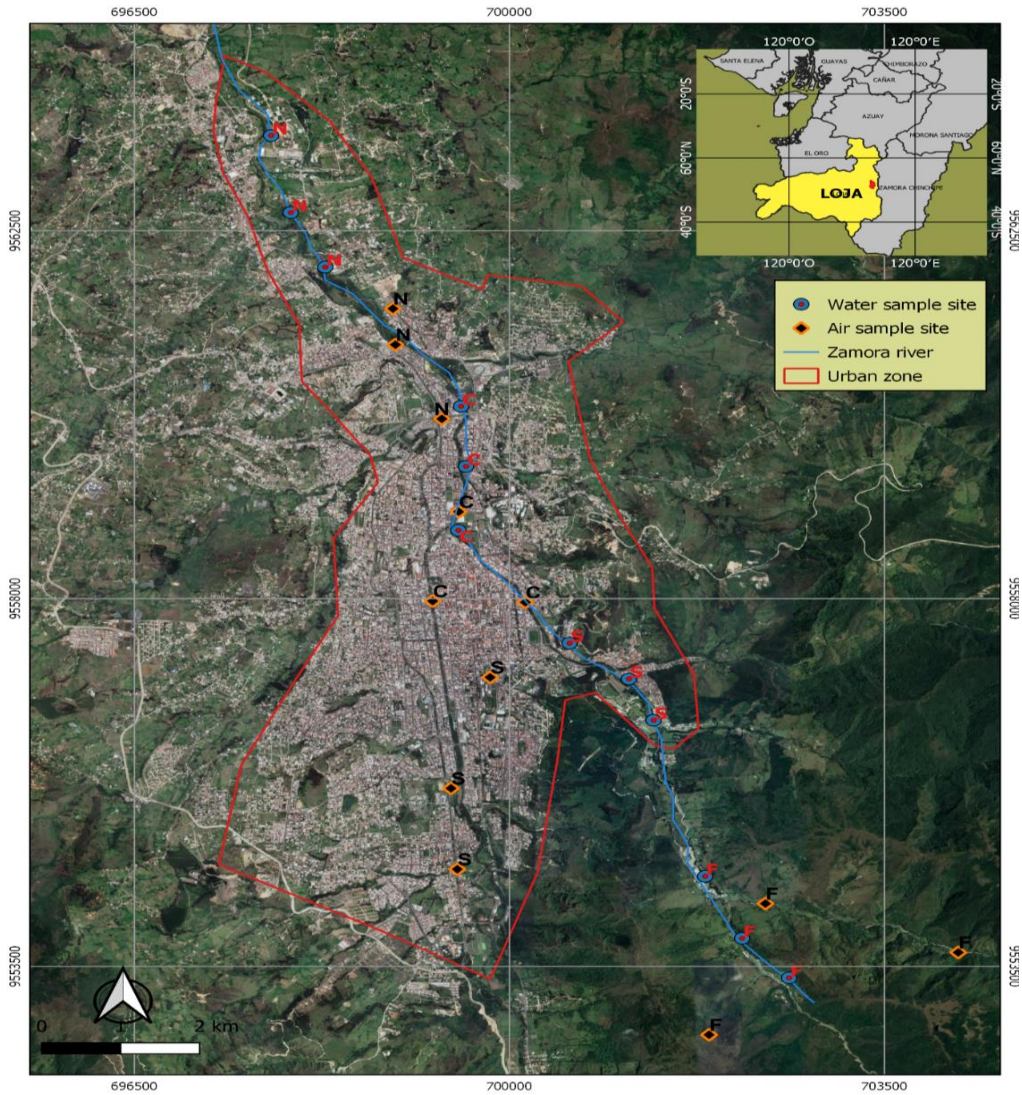

Figure 1. Map of the study area with sampling sites in the city of Loja. The red polygon shown urban zone of the city of Loja.

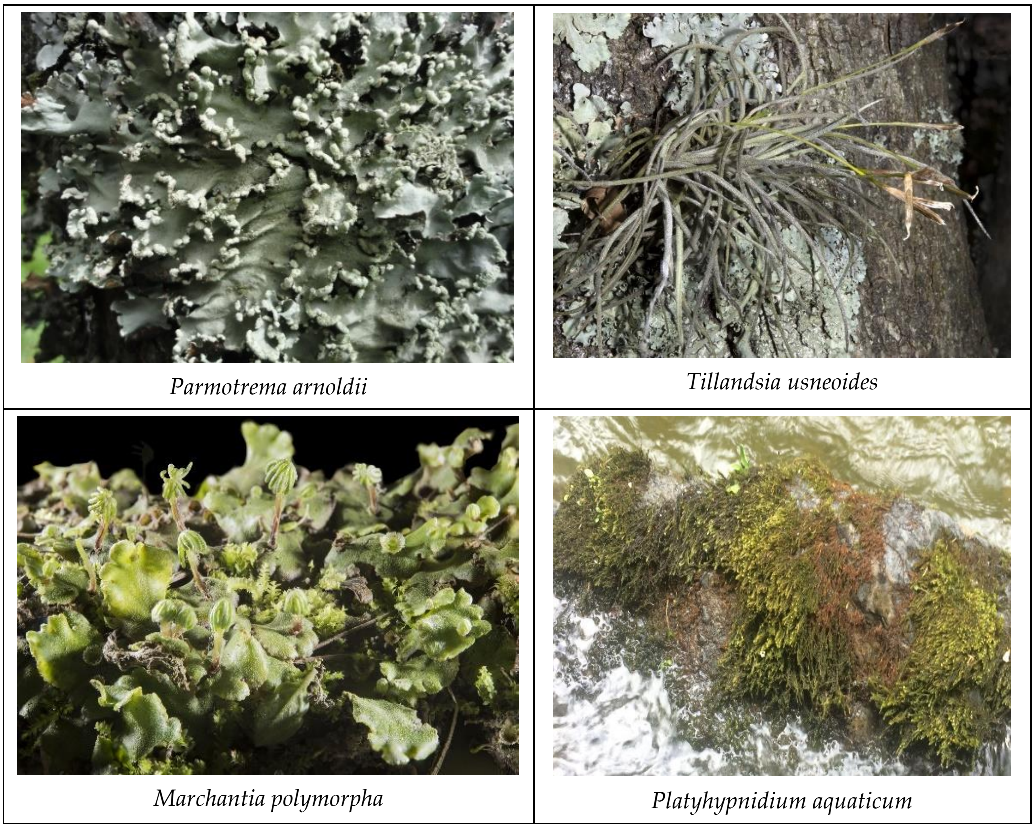

Figure 2. Bryophytes, lichens and bromeliads for evaluating air and water pollution in an Andean city.

2.4. Data Analysis

To assess changes in the concentrations of the metals cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb) and zinc (Zn) between *P. arnoldii* and *T. usneoides*, we used the non-parametric Mann–Whitney U test, due to the fact that the data did not have a normal distribution (Shapiro–Wilk, *p*-valor <0,05). Similarly, changes in the concentrations of cadmium (Cd), manganese (Mn), aluminum (Al), iron (Fe), lead (Pb), zinc (Zn) and arsenic (As) were also assessed between *M. polymorpha* and *P. aquaticum*. All analyses were carried out using RStudio statistical software, version 1.1.453 [58].

3. Results

Metal concentrations in *P. arnoldii* were Zn > Mn > Pb >Cd > Cu, with the maximum values of Mn and Zn (>100 mg/g), and those of Cd, Pb and Cu were lower than 100 mg/g. For *T. usneoides*, metal concentrations were Zn > Mn > Pb > Cd > Cu, with Pb, Mn and Zn exceeding (>100 mg/g) and only Cd and Cu being less than 100 mg/g (Table 1).

Although *Tillandsia usneoides* showed higher mean concentrations than *Parmotrema arnoldii*, the differences were not significant (Figure 3, Table 2). For Mn, the concentration showed significant differences between species (Table 2). Most metals in *P. arnoldii* and *T. usneoides* were much higher for urban areas than the concentrations detected in control areas.

For water quality, *P. aquaticum* showed a higher accumulation capacity compared to *M. polymorpha.* The concentrations of metals in *M. polymorpha* were Al > Zn > Fe > Cd > As > Mn > Pb, with maximum values for Zn, Fe, Al and As higher than 10 μg g^{-1} and those of Mn and Pb lower than 1 μg g^{-1}. For *P. aquaticum*, the bioaccumulation of metals were Al > Mn > Fe > Zn > As > Pb > Cd, where Zn, Fe, Al and Mn exceeded the maximum value of 1000 μg g^{-1} and only the metal Cd showed a value lower than 10 μg g^{-1} (Table 1).

Al, Zn, Fe, As, Pb and Mn showed significant differences between *Marchantia polymorpha* and *Platyhypnidium aquaticum*; however, for Cd, no significant differences were detected (Figure 4, Table 3). The majority of metals in *Marchantia polymorpha* and *Platyhypnidium aquaticum* were much higher for urban areas than the concentrations detected in control areas.

Table 1. Descriptive statistics on heavy metals (mg/g) in *Parmotrema arnoldii* and *Tillandsia usneoides*. Descriptive statistics on heavy metals (μg g^{-1}) in *Marchantia polymorpha* and *Platyhypnidium aquaticum*. M: mean value; SD: standard deviation.

Species	Heavy Metal	Forest	South	Center	North
Parmotrema arnoldii (mg/g)	Cd	0.60 ± 0.805	30.83 ± 19.124	34.66 ± 9.221	27.99 ± 9.058
	Pb	7.14 ± 3.053	39.48 ± 14.659	25.29 ± 9.464	42.95 ± 18.030
	Cu	10.41 ± 7.367	21.27 ± 3.928	25.41 ± 4.441	31.02 ± 4.129
	Mn	12.30 ± 3.430	53.49 ± 18.971	20.03 ± 8.422	56.81 ± 25.589
	Zn	16.19 ± 3.688	91.37 ± 35.781	100.54 ± 23.921	44.46 ± 26.487
Tillandsia usneoides (mg/g)	Cd	1.10 ± 1.272	49.16 ± 6.927	28.93 ± 12.158	28.11 ± 8.170
	Pb	12.29 ± 3.677	35.53 ± 10.754	27.74 ± 14.525	49.93 ± 10.811
	Cu	9.08 ± 5.976	27.57 ± 11.550	22.36 ± 9.685	28.44 ± 5.974
	Mn	15.60 ± 2.522	71.43 ± 34.935	43.27 ± 22.314	96.12 ± 29.603
	Zn	54.65 ± 13.001	70.97 ± 33.702	89.54 ± 24.085	30.11 ± 8.491
Marchantia polymorpha (μg g^{-1})	Cd	0.01 ± 1.742	0.01 ± 001	0.01 ± 010	0.01 ± 003
	Zn	3.08 ± 167	11.64 ± 1.242	13.02 ± 1.901	10.71 ± 0.497
	Fe	0.34 ± 0.342	8.84 ± 1.531	7.61 ± 1.282	6.03 ± 0.583
	Al	7.82 ± 1.741	11.43 ± 2.100	13.21 ± 1.510	8.31 ± 0.874
	Pb	0.04 ± 0.036	0.04 ± 001	0.04 ± 000	0.03 ± 010
	Mn	0.23 ± 0.230	0.75 ± 0.093	0.54 ± 023	0.37 ± 0.077
	As	0 ± 000	0.42 ± 1.120	4.28 ± 4.691	3.94 ± 4.375
Platyhypnidium aquaticum (μg g^{-1})	Cd	0.72 ± 0.751	4.92 ± 1.230	5.25 ± 0.656	6.64 ± 0.806
	Zn	185.08 ± 138.384	913.87 ± 256.436	640.4 ± 245.748	559.55 ± 107.930
	Fe	510.13 ± 164.041	1179.52 ± 301.680	977.03 ± 320.574	1076.6 ± 197.255
	Al	1355.12 ± 343.114	1390.17 ± 671.933	1505.45 ± 407.687	1370.42 ± 516.179
	Pb	10.62 ± 6.970	10.39 ± 8.864	12.86 ± 10.676	12.74 ± 9.675
	Mn	0.4 ± 1.023	2412.21 ± 1301.376	936.49 ± 1032.782	536.62 ± 549.811
	As	6.81 ± 3.585	22.2 ± 5.193	17.25 ± 5.664	21.95 ± 8.190

Table 2. Mann–Whitney U test for heavy metal concentrations in *Parmotrema arnoldii* and *Tillandsia usneoides*.

Metal	W	*p* Value
Cd	1082.5	0.1486
Pb	1497	0.1848
Cu	1326	0.8565
Mn	881	0.0152
Zn	1126	0.2493

Table 3. Mann–Whitney U test for heavy metal concentrations in *Platyhypnidium aquaticum* and *Marchantia polymorpha*.

Metal	W	*p* Value
Cd	1716	0.3576
Zn	254	<0.0001
Fe	350	<0.0001
Al	140	<0.0001
Pb	267	<0.0001
Mn	832	<0.0001
As	182	<0.0001

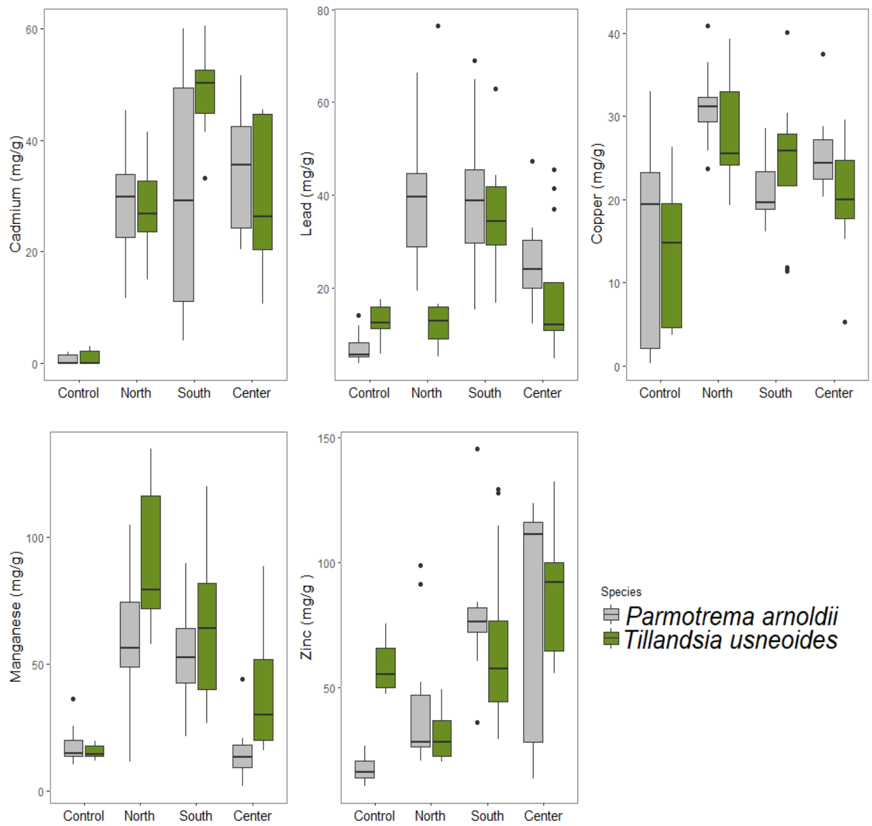

Figure 3. Box plot of metal (Cd, Pb, Cu, Mn and Zn) concentrations (mg/g) in *Parmotrema arnoldii* and *Tillandsia usneoides*, for air quality in different study areas. Boxplots showing the outliers (black circles).

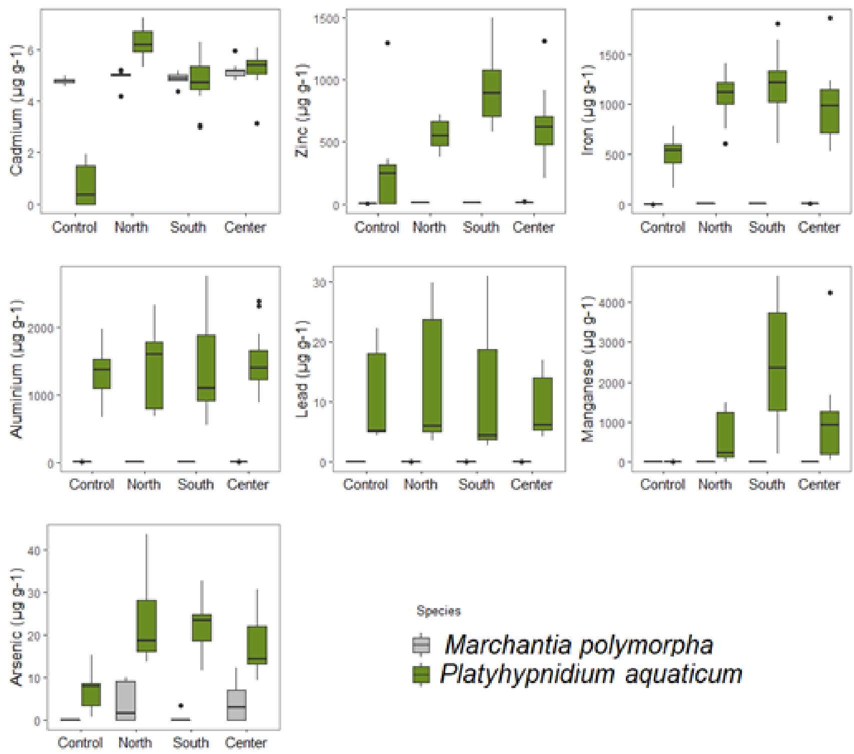

Figure 4. Concentrations (µg g^{-1}) of metals (Cd, Zn, Fe, Al, Pb, Mn and As) in *Marchantia polymorpha* and *Platyhypnidium aquaticum*, for water quality in different study areas. Boxplots showing the outliers (black circles).

4. Discussion

Our results showed that *Parmotrema arnoldii* and *Tillandsia usneoides* have similar heavy metal accumulation related to air quality, as evidenced in other studies [50,51,59,60]. However, for water quality, it was found that the moss *Platyhypnidium aquaticum* had a higher concentration of metals compared to *Marchantia polymorpha,* results corroborating that mosses are more effective than liverworts for monitoring water quality [13,25,42,48,61].

For air quality in the city, no significant differences in cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) concentrations were found between the two species (*Parmotrema arnoldii* and *Tillandsia usneoides*); however, manganese (Mn) showed a slight difference between the species, which may be due to physiological responses, in terms of needs or protection against this element [51]. Pyatt et al. [60] and Monna et al. [51] showed no differences in heavy metal accumulation between the two groups (lichens and bromeliads). Therefore, in a first instance, we can suggest the use of *Parmotrema arnoldii* or *Tillandsia usneoides* for air quality monitoring in other tropical cities. *Parmotrema arnoldii* accumulates more Zn, whereas *Tillandsia usneoides* accumulates more Cd, Pb, Mn and Cu, related to vehicular traffic [18,50].

In this context, previous studies have determined the effectiveness of lichens in accumulating metals Zn, Cd and Pb [23,25,62,63], and also that bromeliads effectively accumulate Cd, Pb and Mn [64–66]. Hence, our results contrast with those found by Safitri et al. [67], Adamo et a.l. [68] and Bargagli et al. [25] claiming that lichens are efficient species in the bioaccumulation of metals, because they are fully exposed to the pollutants. Likewise, we corroborate the statements made by Cardoso-Gustavson et al. [69] and Bermudez et al. [70], who found that *Tillandsia* species accumulate slightly higher concentrations of atmospheric metals than lichens, which may be due to the presence of scales along the leaf surfaces [71]. This morphological adaptation could increase the efficiency of bromeliads to retain metals within their tissue [72,73].

However, regarding water pollution, our results showed that *Platyhypnidium aquaticum* had higher concentrations of zinc, iron, aluminium, lead, manganese and arsenic compared to *Marchantia polymorpha,* which only had higher average concentrations of cadmium. This reflects the ability to bioaccumulate heavy metals and metalloids from *P. aquaticum*, despite the fact that the two species studied have similar habitat requirements [24,47]. These results are congruent with the studies by Kosior et al. [74], Ceschin et al. [45], Puczko et al. [52] and Cesa et al. [75], which show that mosses are important bioindicators in the uptake and accumulation of water pollution, compared to liverworts [21,45,76,77]. We suggest that the presence of these contaminants may be related to water pollution with residual discharges and a lack of treatment systems along urban zones of the river [24,47].

An efficiency advantage of water quality monitoring by mosses is their tolerance to considerable fluctuations in metals, and they are often found fully submerged [78], thus avoiding bioaccumulation or assimilation of other types of elements from the environment. In addition, some studies indicate that *Platyhipnidium* genus is characterized by inhabiting open environments with poor water conditions, which makes them suitable for studies in populated centers [46,74]. Likewise, its wide distribution along river basins makes it suitable for biomonitoring [4,61]. Finally, our study was also consistent with Becerra et al. [79], who reported the efficiency and accumulation capacity of *P. aquaticum* even in areas with low pollution levels.

Even though passive monitoring is effective for monitoring air and water quality, active monitoring overcomes certain limitations of passive monitoring (native species), e.g., active biomonitoring excludes possible phenotypic or genotypic adaptation of native plants and allows temporal interpretation, because the initial concentrations of transplanted elements are known and the duration of exposure to metals can be manipulated [18,21,61,68,80–83]. Finally, it is recommended to take into account biological and population differences and environmental factors related to the site and study organisms, which affect the physiological and biochemical plasticity of the species [15,84].

5. Conclusions

Parmotrema arnoldii and *Tillandsia usneoides* were suitable for monitoring air pollution by heavy metals and both showed similar accumulation capacity. In addition, the moss *Platyhypnidium aquaticum* was more effective in accumulating metals and metalloids than *Marchantia polymorpha,* in relation to water pollution. Bromeliads, lichens and bryophytes provide relevant information on the state of air and water quality, and are reliable and economical tools for establishing the biomonitoring of environmental quality (air and water) in tropical areas.

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