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Lichens and Bromeliads as Bioindicators of Heavy Metal Deposition in Ecuador

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Abstract: We evaluated heavy metal deposition in *Parmotrema arnoldii* and *Tillandsia usneoides* in response to air pollution in Loja city, Ecuador. We assessed heavy metal (cadmium, copper, manganese, lead and zinc) content in these organisms at nine study sites inside Loja city and three control sites in nearby forests. Concentrations of all studied heavy metals (i.e., cadmium (Cd), copper (Cu), lead (Pb), manganese (Mn) and zinc (Zn)) were highest in downtown Loja. Our study confirms that passive monitoring using lichens and/or bromeliads can be an efficient tool to evaluate heavy metal deposition related to urbanization (e.g., vehicle emissions). We recommend these organisms to be used in cost-effective monitoring of air pollution in tropical countries.

Keywords: air pollution; epiphytes; passive monitoring; vehicle emissions

1. Introduction

Air pollution is considered one of the biggest environmental problems in many cities around the world [1], due to increased urbanization, industrial production, rising emissions from traffic and the lack of urban planning [2–4]. Automobiles are one of the major factors, as they emit exhaust and non-exhaust contaminants [5]. Heavy metal deposition is one of the most serious aspects of air pollution. Due to the high toxicity and persistence in the environment, heavy metals have a direct and serious impact on human health [6–8]. In this context, several studies documented that heavy metals, for example, cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) are among the most toxic air pollutants. Furthermore, in urban zones additional anthropogenic sources of heavy metals may include industrial activities, fuel combustion and the production of batteries [9]. For these reasons, implementing accurate and cost-effective air-monitoring strategies is critical to understand how emissions from different sources affect air quality.

Ecuador is among the many countries that suffer from severe air pollution. Recently, substantial population growth in Ecuador has generated increased emissions from industry, increased traffic, and rise in the use of low quality fuels [10,11]. In Loja city, air pollution is currently considered one of the main environmental problems [12]. Nevertheless, to date, only the largest Ecuadorian cities (i.e., Quito, Guayaquil and Cuenca) have established permanent air quality monitoring stations. This follows a general trend throughout Ecuador, where only few studies have reported any effects of fine particulate matter or low air quality [13–15].

Contrary to expectations, air pollution monitoring programs using low-cost biological indicators as an alternative to expensive measuring stations have not been carried out anywhere in the country. This is surprising, as many organisms are cost-effective and efficient indicators of air quality. Among the organisms best suited to this task are bromeliads and lichens, as they obtain all their nutrients directly from the air. Thus, heavy metal content of their biological tissues directly reflects air quality [16,17]. Both types of organisms have been widely used in monitoring schemes in several cities around the globe [18–22]. Lichens, particularly in the family Parmeliaceae (e.g., *Evernia prunastri* (L.) Ach. [23–26], *Flavoparmelia caperata* (L.) Hale [27,28], and *Hypogymnia physodes* (L.) Nyl. [29]), and species in the genus *Parmotrema* A. Massal. [30–35]), are effective indicators of heavy metal deposition. Moreover, epiphytic vascular plants, particularly bromeliads in the genus *Tillandsia* L., are efficient bioaccumulators of heavy metal deposition [16,36–38]. Heavy metal concentrations in the tissues of both *Tillandsia usneoides* and *Parmotrema* ssp. presented a strong correlation with heavy metal deposition from either automobile emissions or industrial sources [9,20,39–43].

Previously, we have used lichens as indicators of air pollution in Ecuador [44]. However, our study evaluated lichen species diversity throughout Loja city as a proxy of air pollution and did not measure heavy metal concentrations [44]. Here, we fill this gap and measure heavy metal concentrations directly in the biological tissues. We show that our technique represents an efficient and cost-effective alternative compared to using expensive measuring devices [45]. We hypothesized that increased urbanization towards the geographic center of the city will result in increased bioaccumulation of heavy metals in *Parmotrema arnoldii* (Du Rietz) Hale and *Tillandsia usneoides* (L.) L.

2. Materials and Methods

2.1. Study Area

Our study area is located in both the urban parts of the city of Loja and the surrounding forests (Figure 1). The mean annual temperature in this region is 20 °C, with annual average rainfalls of ca. 1900 mm, and throughout the year it is characterized by an average relative humidity of ca. 80% (Instituto Nacional de Meteorología e Hidrología, INAMI). Altitude above sea level in the area ranges from 2000 to 2300 m. Three study sites were selected within three zones (South, Center and North) of the city and a control zone (Forests) outside the city, for a total of twelve sites. The three city zones have been shown previously to have high levels of fine particulate matter (PM 2.5), for example, 0.025, 0.05, and 0.038 $\mu g/m^3$ for South, Centre and North zones, respectively [12]. In addition, these city zones are localized in critical points of traffic congestion (738–2791 vehicles) where the concentration of PM 2.5 exceeds the norm (0,015 $\mu g/m^3$) [12]. Additional information on study sites is as follows [12,44,46]:

(1) Forested Zone (F): Our control zone is characterized by fragments of evergreen tropical forest close to the Podocarpus National Park. The area is generally densely vegetated with a low human population and very little rural traffic. This zone presumably acts as an air pollution buffer for the larger area surrounding the city of Loja.

(2) Southern Zone (S): This district is characterized by extensive green areas and recreational parks (1,053,000 m^2), and a low quantity of green area per inhabitant (15.38 m^2/inhabitant), but is nevertheless subject to relatively high traffic due to the transit between this area and the city.

(3) Central Zone (C): The downtown district is a mostly urban area, with a low quantity of green area per inhabitant (11.58 m^2/inhabitant) and very little vegetation (green areas cover only 635,000 m^2); and is subject to high volumes of traffic.

(4) Northern Zone (N): With a relatively large quantity of green space (1,060,000 m^2), this city district has a high amount of green space per inhabitant (38.95 m^2/inhabitant); it is subject to moderate traffic only.

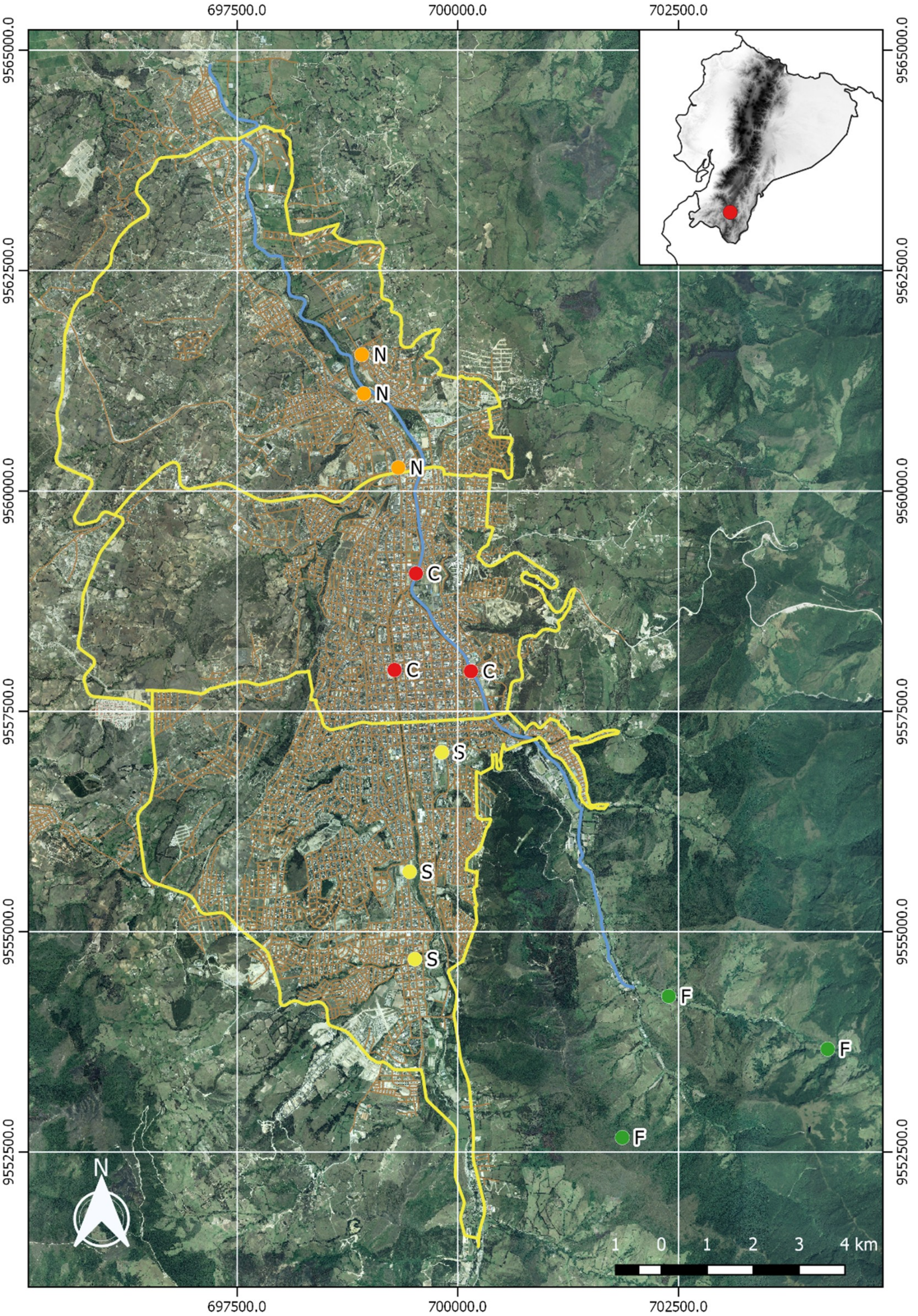

Figure 1. Study area in Loja Province (city of Loja), Southern Ecuador, showing the location of study sites for different levels of air pollution: Forests (F); South (S); Center (C) and North (N).

2.2. Heavy Metal Measures

Within each locality, we collected five samples on different trees (*Salix humboldtiana* Willd. and *Alnus acuminata* Kunth). Each sample consisted of 0.5–1 g of both *Parmotrema arnoldii* and *Tillandsia usneoides*. To assess content of cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb) and zinc (Zn) in these samples, we measured the absorption spectra of five replicas of each sample. Each sample was weighed and digested with 8 ml HNO_3 (70%) and 2 ml H_2O_2 (30%), using a high performance microware system (Milestone SRL., Sorisole (BG), Italy), following the US EPA 3502 method. After digestion, the volume of each sample was adjusted to 100 ml using double deionised water. The content of heavy metal in these samples was then analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 8000; Perkin Elmer). The argon flow rate was adjusted to 12 L/min and air flow rate to 1.2 L/min. Certificated standards (Merk KGaA, Germany) were used for the calibration curves.

Tillandsia usneoides and *Parmotrema arnoldii* were identified using published keys [47,48]. Furthermore, we tested for specific secondary compounds of *Parmotrema arnoldii* using spot tests based on thallus fluorescence under ultraviolet light, with K (10% water solution of potassium hydroxide) and Cl (bleach). The specimens were stored in the Herbarium HUTPL of Universidad Técnica Particular de Loja under *AB 232* museum codes.

2.3. Data Analysis

The effect of zone and species on heavy metal accumulation in *Parmotrema arnoldii* and *Tillandsia usneoides* was modelled by linear mixed models (LMMs). For several heavy metals (zinc), log (x+2) transformations were applied to meet model assumptions. Models were finally checked for residual normality with the Shapiro–Wilk test (p-value > 0.05). The minimal adequate model was selected based on Akaike's Information Criterion (AIC). We used the package 'nlme' [49]. Data were analyzed from a multi-level approach, considering locality as a random factor and introducing the explanatory variables as fixed factors (Zone and species). In the selected model, we evaluated heavy metals accumulation between zone, species, and the interaction between the two using F tests. To identify significant differences in heavy metals accumulation between zones, post-hoc Tukey HSD multiple comparison tests were implemented in the package 'lsmeans'. All analyses were performed using R statistical software version 3.1.13 (R Foundation for Statistical Computing, Vienna, Austria) [50].

3. Results

In *Parmotrema arnoldii*, Cd levels ranged from 0.6 mg/g (Forests) to 34.7 mg/g (Center). Cu levels ranged from 10.41 mg/g (Forests) to 31.02 mg/g (North). Mn levels ranged from 12.30 mg/g (Forests) to 56.81 mg/g (North). Pb levels ranged from 7.14 mg/g (Forests) to 42.95 mg/g (North). Finally, Zn levels ranged from 16.19 mg/g (Forests) to 100.54 mg/g (Center). On the other hand, in *T. usneoides*, Cd levels ranged from 1.1 mg/g (Forests) to 49.2 mg/g (South). Cu levels ranged from 9.08 mg/g (Forests) to 28.44 mg/g (North). Mn levels ranged from 15.60 mg/g (Forests) to 96.12 mg/g (North). Pb levels ranged from 12.29 mg/g (Forests) to 49.93 mg/g (North). Finally, Zn levels ranged from 54.65 mg/g (Forests) to 89.54 mg/g (Center).

At all sites within the Control zone (Forests), *Parmotrema arnoldii* and *Tillandsia usneoides* exhibited a low concentration for all heavy metals examined (Cd, Cu, Mn, Pb) in comparison with the South, Center and North zones (Figure 2a,b; Table A1). However, *T. usneoides* showed a low concentration of Zn in the North compared with the Control zone (Figure 2b). The maximum accumulation of Cd and Zn for the two species was found at the Central zone, followed by the Southern and Northern (Table A1). In the case of zinc, for both *P. arnoldii* and *T. usneoides* the highest concentration was found in the Center zone (100.54 and 89.54 mg/g, respectively), followed by South (91.37 and 70.97 mg/g, respectively) and North (44.46 and 30.11 mg/g, respectively). The level of lead (Pb) was also highest in urban areas for *P. arnoldii* and *T. usneoides*, with the highest levels in the North Zone (42.95 and 49.93 mg/g respectively), followed by South (39.48 and 35.53 mg/g, respectively) and Center (25.29 and 27.74 mg/g, respectively). The highest concentrations of Cu for the two species were recorded in the urban zones (South, Center and North), while the lowest concentration was found at the control site (Forests) (Figure 2a,b; Table A1).

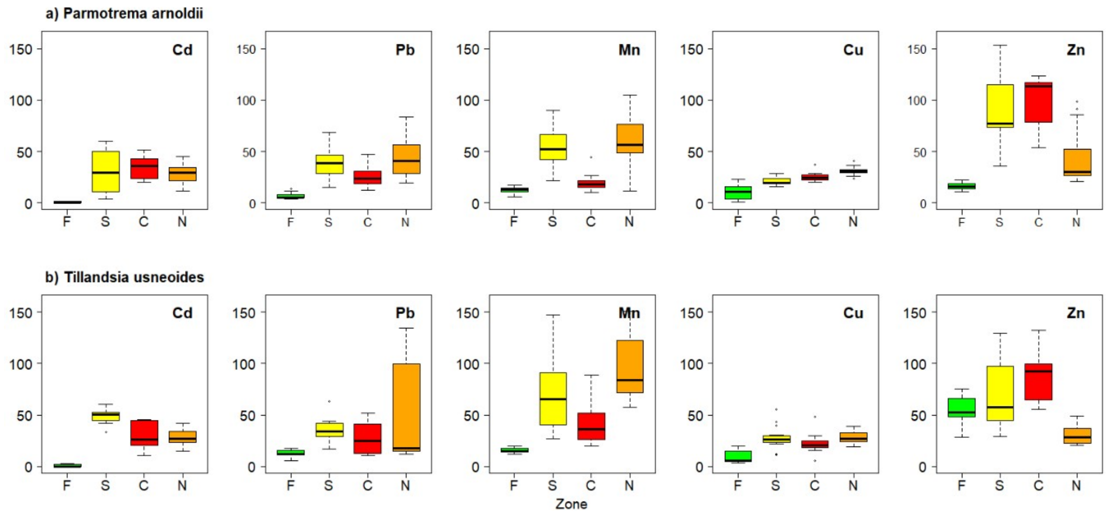

Figure 2. Boxplots depicting heavy metal (Cd, Pb, Mn, Cu and Zn) concentrations in (**a**) *Parmotrema arnoldii*, and (**b**) *Tillandsia usneoides* in Loja. Colours correspond to zones: Yellow = South, orange = North, red = Center, green = Forest.

Results of LMM showed that the concentrations of heavy metals Cd, Cu, Mn, Pb, Zn in *Parmotrema arnoldii* and *Tillandsia usneoides* were significantly different in the four zones. The concentration of these metals in both *Parmotrema arnoldii* and *Tillandsia usneoides* significantly decreased in control zones where air pollution and, thus, heavy metal deposition was lower (Figure 2a,b; Table 1).

Table 1. Linear mixed model (LMM) and a post-hoc Tukey test of heavy metal accumulation in *Parmotrema arnoldii* and *Tillandsia usneoides* according to the different study sites. *F-value* = statistical; $p < 0.05$ is considered significant.

LMM	Cd		Pb		Mn		Cu		Zinc	
Factor	**F**	***p*-Value**	**F**	***p*-Value**	**F**	***p*-Value**	**F**	***p*-Value**	**F**	***p*-Value**
Zone	51.84	**<0.001**	26.37	**<0.001**	33.48	**<0.001**	28.12	**<0.001**	53.35	**<0.001**
Specie	3.80	**0.054**	0.005	0.944	22.17	**<0.001**	0.03	0.863	0.051	0.822
Zone x Specie	6.27	**<0.001**	1.061	0.37	2.32	0.08	2.457	0.067	15.67	**<0.001**

Tukey's HSD Test	Cd		Pb		Mn		Cu		Zinc	
Zone	**Est**	***p*-Value**	**Est**	***p*-Value**	**Est**	***p*-Value**	**Est**	***p*-Value**	**Est**	***p*-Value**
F - S	−38.79	**<0.001**	−0.865	**<0.001**	16.58	0.419	−14.68	**<0.001**	−0.73	**<0.001**
F - C	30.54	**<0.001**	−0.583	**<0.001**	−61.38	**0.001**	−14.14	**<0.001**	−0.92	**<0.001**
F - N	−26.99	**<0.001**	−0.891	**<0.001**	−47.00	**0.004**	−19.98	**<0.001**	−0.08	0.823
S - C	−8.25	0.185	0.282	**0.039**	−30.41	**0.042**	0.53	0.993	−0.19	0.132
S - N	−11.79	**0.043**	−0.026	0.994	14.38	0.457	−5.30	**0.045**	0.652	**<0.001**
C - N	3.54	0.798	−0.309	**0.028**	−44.79	**0.006**	−5.84	0.033	0.841	**<0.001**

The interaction between zone and species was only significant for Cd and Zn, and species showed an effect on concentration of Cd and Mn (Table 1). The Tukey HSD test showed significant differences according to zone between the heavy metal accumulation of forests (control zone) and the three urbanized zones (South, Center and North; Table 1).

4. Discussion

Our results demonstrate that heavy metal concentrations in *Parmotrema arnoldii* (a lichen) and *Tillandsia usneoides* (a bromeliad) closely reflected heavy metal deposition in Loja city. We hypothesized that heavy metal accumulation in *P. arnoldii* and *T. usneoides* may be responding to automobile traffic contamination. Previous research has found a high correlation between heavy metals in air and automobile traffic in the urban parts of Loja [12,46]. A similar pattern has been found in many other areas of the world, that is, that air pollution caused by heavy metal deposition generally tends to be higher in urban zones with more traffic, than in rural areas with less traffic [1,16,26,33,51]

The accumulation of Cd, Cu, Pb and Mn in *Parmotrema arnoldii* and *Tillandsia usneoides* tissues showed a similar pattern, with more heavy metals in urban areas than in nearby forest controls. The strong enrichment of heavy metals at the urban sites of Loja is not unexpected, particularly enrichment of lead from particle deposition as a result of an increased volume of traffic [12,46]. Our findings are consistent with Monna et al. [52], who found high enrichment of Cd, Cu, Mn and Pb in urban areas for *Parmotrema crinitum* and *Tillandsia usneoides*. Following a similar pattern, Figueiredo et al. [20] also reported high concentrations of heavy metal in urban zones for *Tillandsia usneoides*. In addition, Sánchez-Chardi [9] demonstrated that five different species of *Tillandsia* accumulated Pb and Cd according to traffic intensity in Asunción, Paraguay, reporting particularly high levels for Pb from all five species obtained from the most polluted areas. Several studies showed that these pollutants (e.g., cadmium, copper, lead and manganese), are related not only directly with exhausts emissions from road traffic caused by fuel and lubricant combustion, but also indirectly from catalytic converters, particulate filters, resuspension, lubricating oils, engine corrosion and wear and tear of tyres [53–58]. Other potential sources of heavy metals can generally be related to metal extraction, industrial uses, waste incineration and oil combustion [59]. However, Loja does not have big industries, which could otherwise contribute to higher levels of background pollution. This might be different for much larger cities of Ecuador, such as Guayaquil, Quito or even Cuenca, where heavy metal emissions from traffic may not be the only pollution source.

Our results agree well with previous studies that used other species of *Parmotrema* (e.g., *Parmotrema chinense*, *Parmotrema crinitum*, *Parmotrema reticulatum* and *Parmotrema tinctorum*), and with several studies that used bromeliads like *Tillandsia usneoides* to indirectly measure heavy metal deposition. All these studies showed similar patterns, where urban zones have high concentrations of Cd, Cu, Pb and Mn [20,21,33,35,52]. However, the concentrations of these heavy metals in our control site (Forest) were relatively low [9,41,42,60]. This would suggest that the overall background contamination by heavy metals in Ecuador might generally be quite low; thus, the air in rural areas goes largely unaffected and an increased concentration is present only in the city itself [46].

We found that heavy metal deposition in *Parmotrema arnoldii* and *Tillandsia usneoides* varied within the city, as compared to control sites (Forest). Relatively high values of zinc (Zn) were detected in specimens of *Parmotrema arnoldii* in somewhat more urbanized zones (Center and South). This is possibly a result of collecting the specimens along bus lines. Similarly, Giordano et al. [61], Aprile et al. [33], and Rhzaoui et al. [26] also found zinc deposition in lichens typically related to increases of traffic along traffic routes serving inner city urban areas. In addition, several studies found higher concentrations of zinc in specimens of genus *Parmotrema* at urban zones with high levels of vehicular traffic [21,33,35,50]. Air pollution from Zn can typically be ascribed to tyre wear, and this metal is also a common component of antioxidants used as dispersants to improve lubricating oils [62]. Thus, the main sources of Zn are indirect emissions oftraffic, not only directly from the exhaust, but also from industrial sources [63]. A comparatively low accumulation of Zn was found in specimens of *Tillandsia usneoides*, particularly in the North and Forest control site, especially in comparison with the other, more urban sites. This was probably due to a buffering effect present in the North, where some of the collection sites are characterized by stands of mixed forests. Ochoa-Jimenez et al. [44] also found less air pollution in similar areas characterized by small mixed forest fragments within urban areas but away from the city center. The sites that they studied resemble some of our sample sites in the Northern zone.

In conclusion, lichens and bromeliads closely correspond with heavy metal deposition likely related to increased traffic emissions in the urban parts of Loja. Our study demonstrates that *Parmotrema arnoldii* and *Tillandsia usneoides* are both suitable and cost-effective indicators of heavy metal deposition in Loja. Thus, we recommend using these lichens and bromeliads to monitor air pollution. We acknowledge that heavy metal measurements from specimens only represent an indirect approximation to the accumulation of heavy metals on a daily basis. Lichens and bromeliads cannot necessarily replace more sophisticated technologies to measure heavy metal deposition directly. Nevertheless, in a relatively small city like Loja, which lacks expensive equipment to measure air pollutants directly, assessing overall contamination using bromeliads and lichens as bioindicators may be an attractive and cheap alternative. In our opinion, it is imperative to establish a more robust, long-term monitoring scheme using both lichens and bromeliads as bioindicators.

Author Contributions: Conceptualization, Á.B. and J.C.; methodology, A.B., J.M., C.V., T.L., Y.L. and J.C.; formal analysis, A.B. and J.C.; investigation, A.B.; resources, A.B. and J.C.; data curation, A.B.; writing—original draft preparation, A.B.; writing—review and editing, A.B. and J.C.; project administration, A.B.; funding acquisition, A.B. and J.C.

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

Appendix A

Table A1. Mean concentration and standard error of Cd, Cu, Mn, Pb and Zn in *Parmotrema arnoldii* (PA) and *Tillandsia usneoides* (TU) from the Loja city (mg/g).

Species	Heavy Metal	Forest	South	Center	North
Parmotrema arnoldii	Cd	0.60 ± 0.81	30.83 ± 19.12	34.66 ± 9.22	27.99 ± 9.06
	Cu	10.41 ± 7.37	21.27 ± 3.93	25.41 ± 4.44	31.02 ± 4.13
	Mn	12.30 ± 3.43	53.49 ± 18.97	20.03 ± 8.42	56.81 ± 25.59
	Pb	7.14 ± 3.05	39.48 ± 14.66	25.29 ± 9.46	42.95 ± 18.03
	Zn	16.19 ± 3.69	91.37 ± 35.78	100.54 ± 23.92	44.46 ± 26.49
Tillandsia usneoides	Cd	1.10 ± 1.27	49.16 ± 6.93	28.93 ± 12.16	28.11 ± 8.17
	Cu	9.08 ± 5.98	27.57 ± 11.55	22.36 ± 9.69	28.44 ± 5.97
	Mn	15.60 ± 2.52	71.43 ± 34.94	43.27 ± 22.31	96.12 ± 29.60
	Pb	12.29 ± 3.68	35.53 ± 10.75	27.74 ± 14.53	49.93 ± 10.81
	Zn	54.65 ± 13.00	70.97 ± 33.70	89.54 ± 24.09	30.11 ± 8.49

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