# Using *Tillandsia usneoides* as Biomonitor for Toxic Metals in the Air of Guanajuato's Tunnel Network

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Guanajuato town is an important touristic center of Mexico, and one of its main attractions is its tunnel network (subterranean streets). In this work the air quality inside this tunnel network was evaluated using samples of *Tillandsia usneoides* as a biomonitor. Twenty-one plastic passive samplers, containing 50 g of plants each, were installed at different points of this tunnel network. In addition, surface layer of the tunnel walls, close to the plant sampling sites, were collected. Estimates for the enrichment factor, using Fe as reference and standard metal crustal data, suggest that the origin of the suspended metals is probably geogenic for Cr, a mix for Cu, and anthropogenic for Zn and Hg. It is important to emphasize that the Guanajuato's tunnel network is used both by pedestrians and vehicular traffic and, probably, the last one is acting as the main source for toxic elements for the tunnel's air.

Graphical abstract
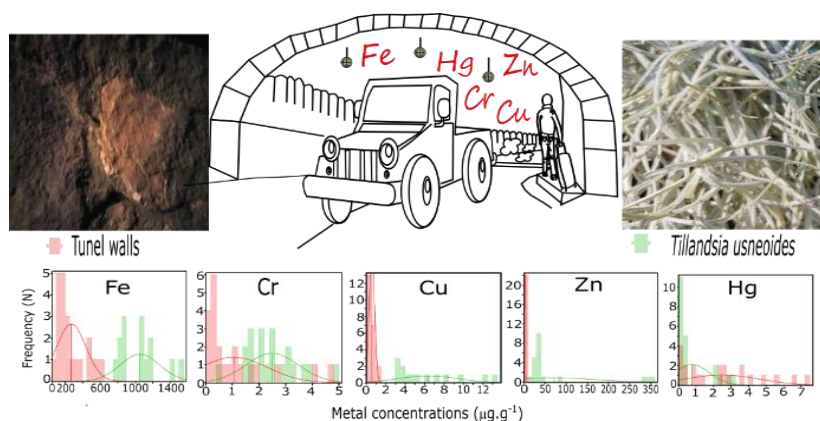
1. Introduction

Guanajuato is a historical town located in the Mexican central plateau. Its historical importance comes mainly from its preserved baroque and neoclassical architecture from a time of expressive gold and silver exploration, beginning during the colonial period (first discover of silver ore in 1548) [1]. Because of this, the UNESCO named it as a World Heritage Site, which attracts a big flux of national and international tourism. One of the main attractions of the city is the network of 'subterranean streets' (tunnels), used both for the vehicular transport and pedestrian circulation. In the present work, we

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carried out an evaluation of the quality of indoor air in this tunnel network.

It is well known that the suspended particulate matter (PM) of mining regions may contain some metals [2]. Toxic elements are persistent in the environment, and, in high concentrations, these elements are considered harmful to plants, humans and other animals. The main sources for the toxic elements atmospheric input are industrial activities, combustion of fossil-based fuels, fuel and lubricant additives, and erosion from distinct sources [2, 3, 4]. Usually, the PM is quantified using automatic sampling instruments integrated in an atmospheric monitoring station; these filters are used to evaluate the atmospheric contamination by specific compounds, such as dioxins, hydrocarbons *etc*, and toxic elements [4]. Since these samplers are expensive, and there is the need for many sampling points to evaluate the atmospheric contamination by a specific chemical, an alternative for sophisticated air samplers is the use of bioindicators. The latter have, as advantages, the low cost, non-dependence on power supply, and easy sampling and handling techniques [5].

Different biological organisms have been used as atmospheric bioindicators, such as lichens, bryophytes, bromeliads, tree barks, *etc* [5, 6, 7]. Among these, the *Tillandsia usneoides*, also known as "Spanish moss", an aerial epiphytic bromeliad that lives on trees or other kinds of inert subtracts, stands out. As all *Crassulaceae* members, the *T. usneoides* utilize the crassulacean acid metabolism to reduce water loss, making it a very adapted specie to survive in extreme dryness. The main adaptations for arid conditions of this plant are: reduced or absent roots (fundamentally used for fixation), and stem and leaves completely covered by scales, which protect the stomata from desiccation. Furthermore, the *T. usneoides* are notably hygroscopic, and the water and nutrients are obtained directly by absorption from aerosols. These characteristics make the *T. usneoides* a very efficient bioindicator to monitor air pollution, including indoor air quality [8, 9]. For instance, it has been used to evaluate the atmospheric contamination by organic compounds (PCBs, HPA, dioxins, DDTs) [10], mercury [11, 12] and other metals [11, 12, 13, 14]. Also, the use of plants as bioindicator has an additional advantage because it is possible to install many sampling points simultaneously, thus enlarging the sample area of study.

Concerning the ability of *T. usneoides* to absorb trace metals from the air, it has been proposed that their foliar trichomes can retain most metals bound to PM [15], acting together with the cuticular waxes as the main uptake via for them [16]. A relationship between PM deposition on the surface of these plants and the measured metal content was confirmed by some authors (*e.g.* [15, 16, 17, 18]). Fonseca *et al.* (2007) demonstrated that metal concentrations adsorbed on *T. usneoides* were proportional to those obtained with a traditional vapor camera (in stable atmosphere conditions and dust free air), with a linear correlation (R = 0.99; p < 0.05) between the Hg measured on the plants (Hg_{TU}, in $\mu g.g^{-1}$) compared to the results on Hg air concentrations in vapor camera (Hg_{VC}, in $\mu g.m^3$) of the form: $Hg_{VC} = 125\ Hg_{TU} - 4.375$ [8].

The main objective of this work was to determine the presence of toxic elements in indoor air along Guanajuato's tunnel network using *T. usneoides* as biomonitor. In the global scenario, this contributes to better characterize the behavior and usefulness of this bromeliad as an effective biomonitor, and to contribute with new measurements that can be compared to the ones from analogous places around the world. Additionally, these results could serve as instruments for the local population to request policies that could improve public health concerning air quality.

2. Results and Discussion

The measurements obtained for the toxic elements in the *T. usneoides* samples and for the wall scrap samples are presented, respectively, in Tables 1 and 2. In both tables, the samples are grouped according to the specific tunnel section where they were collected.

Table 1. Toxic elements concentration ($\mu g.g^{-1}$ dry weight) in the *Tillandsia usneoides* samples installed along the Guanajuato's tunnel network[a]. Uncertainties are the SD for the 3 replicates.

Hg	Cr	Cd	Zn	Fe	Cu
Controls (before exposition)					
0.09 ± 0.01	0.30 ± 0.04	0.09 ± 0.01	2.54 ± 0.20	93.4 ± 2.6	1.01 ± 0.36
***Ángeles* (An), 550m long**					
2.92 ± 0.00	3.78 ± 0.87	0.15 ± 0.01	26.8 ± 1.1	1158 ± 104	7.29 ± 0.66
2.03 ± 0.37	4.95 ± 1.15	0.10 ± 0.03	35.6 ± 0.4	1443 ± 22	12.22 ± 0.23
***El Barretero* (EB), 800m long**					
2.03 ± 0.01	3.12 ± 0.06	0.21 ± 0.05	31.7 ± 1.8	1546 ± 139	13.30 ± 1.22
0.18 ± 0.01	2.13 ± 0.02	0.27 ± 0.02	38.8 ± 0.6	816 ± 3	3.85 ± 0.02
***Santa Fé* (SF), 450m long**					
0.21 ± 0.02	1.71 ± 0.09	0.60 ± 0.08	33.6 ± 0.3	831 ± 94	ND
0.22 ± 0.03	1.55 ± 0.19	0.42 ± 0.09	29.4 ± 2.1	619 ± 83	ND
0.24 ± 0.02	2.10 ± 0.20	0.34 ± 0.15	87.3 ± 14.9	886 ± 104	ND
***Miguel Hidalgo y Costilla* (MH), 1500m long, 7 ventilation gaps**					
0.23 ± 0.02	2.38 ± 0.06	0.20 ± 0.00	31.5 ± 0.2	894 ± 26	4.75 ± 0.05
0.16 ± 0.16	2.93 ± 0.18	0.23 ± 0.02	40.0 ± 0.2	1063 ± 1	9.77 ± 0.43
0.20 ± 0.02	2.12 ± 0.37	0.15 ± 0.01	31.3 ± 0.4	1081 ± 434	3.49 ± 0.60
0.20 ± 0.00	1.70 ± 0.05	0.16 ± 0.02	35.1 ± 0.8	1099 ± 30	6.17 ± 0.14

Ponciano Aguilar (PA), 1100m long, 3 ventilation gaps					
0.37 ± 0.00	3.30 ± 0.23	0.17 ± 0.00	304.7 ± 1.6	1106 ± 12	5.02 ± 0.02
0.26 ± 0.04	2.60 ± 0.06	0.25 ± 0.05	35.3 ± 0.4	885 ± 53	3.42 ± 0.15
0.22 ± 0.02	1.65 ± 0.04	0.11 ± 0.02	27.0 ± 0.4	782 ± 4	3.76 ± 0.05
Tamazuca (Tz), 125m long					
0.31 ± 0.03	3.05 ± 0.09	0.21 ± 0.03	296.2 ± 2.9	868 ± 6	4.33 ± 0.04
0.23 ± 0.00	2.44 ± 0.17	0.33 ± 0.19	289.2 ± 3.6	820 ± 23	3.42 ± 0.24
Tiburcio Ávarez (TA), 900m long					
0.17 ± 0.00	0.56 ± 1.02	0.34 ± 0.17	28.3 ± 0.2	1179 ± 10	ND
2.33 ± 0.06	2.41 ± 0.17	0.19 ± 0.02	30.4 ± 1.2	542 ± 7	ND
0.27 ± 0.00	1.47 ± 0.09	0.32 ± 0.12	35.8 ± 0.2	872 ± 2	ND
0.25 ± 0.01	ND	1.89 ± 0.08	12.6 ± 19.3	265 ± 37	ND
Detection Limits					
0.07	**0.01**	**0.003**	**0.1**	**0.25**	**0.02**
São Paulo city, Brazil [b]					
-	1.0−3.7	-	41−189	612−1324	4.3−56
Mezquital Valley, Mexico [c]					
-	0.8−40.4	0.9−6.5	4−192	401−4368	2−48.4

Notes to table. (a) ND: not determined. All samples showed Pb concentration below DL (0.0072 µg.g⁻¹). Reference data: (b) Figueiredo *et al.* (2007) [19] and (c) Zambrano-García *et al.* (2009) [20].

Table 2. Toxic elements concentration (µg.g⁻¹ dry weight) in wall surface samples of Guanajuato's tunnel network. Uncertainties are the SD for the 3 replicates.

Hg	Cr	Zn	Fe	Cu	Pb	Mn
Ángeles (An), 550m long						
2.31 ± 0.25	1.72 ± 0.04	0.73 ± 0.07	151 ± 44	0.25 ± 0.00	0.77 ± 0.06	9.91 ± 1.07
0.22 ± 0.01	0.05 ± 0.01	0.41 ± 0.01	262 ± 28	0.61 ± 0.05	0.72 ± 0.45	2.58 ± 0.05
3.51 ± 0.26	0.36 ± 0.05	3.90 ± 0.00	168	1.0 ± 0.01	2.44 ± 0.68	4.87 ± 0.03
El Barretero (EB), 800m long						
0.47 ± 0.04	0.67 ± 0.10	1.16 ± 0.14	527	0.39 ± 0.01	1.03 ± 0.05	12.78 ± 1.96
4.89 ± 0.01	1.16 ± 0.01	5.10 ± 0.50	629	0.81 ± 0.05	5.47 ± 0.98	12.34 ± 0.13
0.88 ± 0.04	0.16 ± 0.02	1.18 ± 0.05	122 ± 16	0.47 ± 0.01	0.83 ± 0.21	2.98 ± 0.15
Santa Fé (SF), 450m long						
7.31 ± 1.20	2.75 ± 0.15	1.71 ± 0.09	390 ± 21	0.92 ± 0.22	2.16 ± 0.40	8.83 ± 0.74
3.52 ± 0.00	1.81 ± 0.06	1.71 ± 0.06	242 ± 32	0.57 ± 0.05	1.71 ± 0.25	4.53 ± 0.49
2.27 ± 0.15	0.17 ± 0.04	1.51 ± 0.15	74 ± 7	0.48 ± 0.04	1.92 ± 0.02	1.64 ± 0.35
2.73 ± 0.30	1.25 ± 0.26	2.10 ± 0.17	325 ± 18	1.33 ± 0.02	2.63 ± 0.20	7.89 ± 0.71
Miguel Hidalgo y Costilla (MH), 1500m long, 7 ventilation gaps						
5.52 ± 0.21	0.30 ± 0.02	1.97 ± 0.01	175 ± 25	0.38 ± 0.01	0.81 ± 0.31	3.83 ± 0.24
6.61 ± 0.11	0.27 ± 0.01	1.99 ± 0.02	118 ± 17	0.82 ± 0.01	1.98 ± 0.08	7.41 ± 0.21
2.98 ± 0.13	0.22 ± 0.07	1.02 ± 0.08	143 ± 58	0.34 ± 0.04	0.94 ± 0.00	2.97 ± 0.21
Ponciano Aguilar (PA), 1100m long, 3 ventilation gaps						
0.46 ± 0.00	4.04 ± 0.01	1.05 ± 0.13	492 ± 6	0.43 ± 0.07	0.93 ± 0.63	9.02 ± 0.66
0.19 ± 0.01	0.34 ± 0.04	3.51 ± 0.12	166 ± 11	0.34 ± 0.02	2.14 ± 0.14	2.93 ± 0.22
0.21 ± 0.00	0.43 ± 0.14	1.25 ± 0.07	203 ± 19	0.49 ± 0.03	1.57 ± 0.44	4.92 ± 0.42
0.76 ± 0.01	1.05 ± 0.06	1.13 ± 0.01	196 ± 15	0.38 ± 0.02	1.15 ± 0.06	3.53 ± 0.20
Tamazuca (Tz), 125m long						
1.28 ± 0.10	0.16 ± 0.07	1.42 ± 0.66	139 ± 58	0.34 ± 0.07	0.40 ± 0.05	3.60 ± 1.33
1.05 ± 0.05	0.28 ± 0.06	0.93 ± 0.04	135 ± 46	0.22 ± 0.01	0.90 ± 0.10	8.34 ± 1.17
Tiburcio Álvarez (TA), 900m long						
0.16 ± 0.01	4.73 ± 0.23	0.78 ± 0.00	596 ± 65	0.32 ± 0.03	1.10 ± 0.15	7.49 ± 0.62
2.70 ± 0.07	0.92 ± 0.11	1.06 ± 0.22	478 ± 29	0.87 ± 0.01	1.96 ± 0.43	8.19 ± 0.21
4.24 ± 0.45	0.40 ± 0.08	2.33 ± 0.09	210 ± 8	0.78 ± 0.04	2.55 ± 0.19	4.07 ± 0.36
Detection Limits						
0.53	**0.01**	**0.01**	**0.4**	**0.01**	**0.07**	**0.003**

It is worth noting that *Santa Fé* (SF), *Ángeles* (An) and *Tamazuca* (Tz) are the most walkable by pedestrians, followed by *Tiburcio Álvarez* (TA) and *El Barretero* (EB). *Miguel Hidalgo y Castilla* (MH) is also much walkable, but it differs from the others because it is a subterranean street with many gaps opened to the free atmospheric air. On the other hand, in this tunnel, people usually stand still for some time because there are many bus stops along it. *Ponciano Aguilar* (PA) also has open parts, but this is less walkable, and all its bus stops are in these gaps. *Tamazuca* is the shortest among the tunnels, with only 125 m extension.

The distribution of measured values for Fe, Cr, Zn and Cu (**Fig. 1**, first four panels) follows the same pattern when we compare the two sample sets: toxic elements in *T. usneoides* (air) show higher values than the ones in the walls (crust). Specifically, the median (and standard deviation) values for air

samples are: 886±289, 2.40±1.01, 33.60±95.03 and 4.89±3.35 ($\mu g.g^{-1}$ dry weight), and for the walls: 200±170, 0.42±1.28, 1.34±1.13 and 0.48±0.29, respectively for Fe, Cr, Cu and Zn. The more skewed values were found for Cr, for which we found few small concentrations in the air samples (of *Tiburcio Álvarez* tunnel) and few large values in the wall samples (*Ponciano Aguilar* and *Tiburcio Álvarez* tunnels). It may be noted that these are only indicative comparisons since plants accumulate actively and walls passively. In fact, they also represent different exposure times, the walls corresponding to elements accumulated certainly before the sampling time of the experiment.
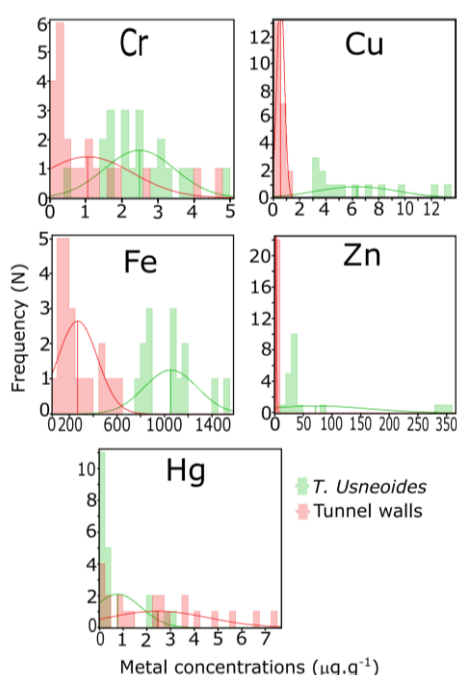
Fig. 1. Histograms of the measured concentrations for Fe, Cr, Zn, Cu and Hg; green histograms are for *T. usneoides* and red histograms for the walls. A Gaussian was plotted above each distribution.

This was not the case for Hg concentration (**Fig. 1**, last panel): the measured values form two groups in both sets of samples, that is, there are both low (lower than 1.5 $\mu g.g^{-1}$) and high values (higher than 2.0 $\mu g.g^{-1}$) of concentration, both in the tunnels air and walls. Considering the global distributions, the measured values were higher for the walls (medians and standard deviations 2.29±2.17 $\mu g.g^{-1}$ compared to 0.24±1.02 $\mu g.g^{-1}$ for the *T. usneoides*).

Comparing the values we measured with the ones observed in other sites [19, 20], we can see that the range of Fe concentrations we found for the *T. usneoides* tissues (542−1546 $\mu g.g^{-1}$) is similar to the one found in the air of big cities like São Paulo and Mexico City (CDMX) surroundings, although this metal is not dangerous for human health. For Zn and Cu, we had ranges of (13−305 $\mu g.g^{-1}$) and (3.4−13.3 $\mu g.g^{-1}$), respectively, also in same ranges of the big cities. The exceptions are the highest values of Zn found in *Ponciano Aguilar* and *Tamazuca*, which are about 50% higher than the maximum ones found in São Paulo city (189 $\mu g.g^{-1}$) and Mexico City (192 $\mu g.g^{-1}$), examples of the worst air qualities in the world. This contamination is probably associated to urban vehicular traffic − these tunnels present a very heavy vehicular traffic, including municipal buses [20]. Suspended particulate matter usually washed out by rain remain inside tunnels with a much higher residence time.

The observed concentrations of Cr in the air (0.56−4.95 $\mu g.g^{-1}$) were similar to the ones in São Paulo city reported range, and smaller than the ones in Mezquital Valley (see **Tab. 1**). This is not the worst scenario since Guanajuato state is known to have a serious problem of Cr contamination [21, 22, 23].

For Hg air concentrations, reference values for *T. usneoides* go from (0.1−0.4 $\mu g.g^{-1}$) in controls to (1.9−26.8 $\mu g.g^{-1}$) in gold trade shops in Amazonian cities [11]. Comparatively, the range we measured in *T. usneoides* samples (0.2−3.2 $\mu g.g^{-1}$) is not extremely high. The highest values were found in *Ángeles* tunnel (2.0−3.2 $\mu g.g^{-1}$), but also in one sample of *El Barretero* and another of *Tiburcio Álvarez*. The highest values in the walls, on the other hand, were found in *Santa Fé* (2.3−7.3 $\mu g.g^{-1}$) and *Miguel Hidalgo y Costilla* (3.0−6.6 $\mu g.g^{-1}$) tunnels, while these tunnels did not show high values in the air (all measured values below 0.25 $\mu g.g^{-1}$). Since concentration in walls was high in almost all tunnels (except for *Ponciano Aguilar*), one could expect that the source for the Hg in the air in these tunnels would be the local mineralogical composition. However, original Hg concentration in the Guanajuato veins was estimated to be less than 1 $\mu g.g^{-1}$ [24, 25]. The explanation for the high values of Hg in the walls of the *Miguel Hidalgo y Costilla* tunnel could be, then, attributed to the Hg used in historical metal mining during the Spanish Colonial era −this subterranean street was originally the channel of a river (now intubated deeper below the tunnel). This could also be the source of Hg for the air in other tunnels of Guanajuato. It is worth noting that, traditionally, the air quality assessment is performed from the particle matter collected with HiVol samplers, while we do it by particles adsorbed to the plants, therefore, it is not possible to make a direct comparison between our data and those obtained with such samplers. Nevertheless, as a comparison, atmospheric Hg concentration values obtained for various countries are cited in Kim et al, 2016 [26]: Canada ranged in 0.54−6.43, China in 1.12−9.49, and Korea in 0.10−25.40 $ng.m^{-3}$, while general values for Northern Hemisphere were found to be inside the 0.71−3.82 range, and for the Southern Hemisphere inside 0.37−3.97 $ng.m^{-3}$. It is important to highlight the relevance of making this kind of data available, which in addition to inferring the air quality to which the population of Guanajuato town is exposed, can support other similar studies, that is, those that use plants (bromeliads) as bioindicators.

In first approximation, the high values of metal concentrations detected on the plant samples (except for Hg) compared to the ones in tunnel walls (**Tab. 1** and **2**, respectively) suggest vehicles as main source of metal for the indoor air, discarding the natural composition of the tunnel's rocks as origin. The argument in the last paragraph would suggest the same for the mercury.

As described above, the use of plants as bioindicators of air metal pollution has become a viable alternative to the traditional HiVol PM sampling technique. While Zheng *et al.* (2020) suggested that, for the *Tillandsia* species, the foliar trichomes can retain most trace metals bound to PM [15], Kim et al. (2020) proposed the trichomes and cuticular waxes as the main responsible uptake via [16]. Concerning specific metals, Amado Filho *et al.* (2002) verified that the Hg adsorbed by *T. usneoides* was retained mainly on the stains and leaves, and less in epidermal cells, while *T. brachycaulos* accumulated metals (Cu, Ni, Zn, and Cr) preferentially within the cell walls, suggesting it as a possible mechanism to prevent contaminants from entering the protoplasm, acting as a barrier [14, 27].

Origin of the toxic elements

The calculated *EF* values for Hg, Cu, Zn and Cr are displayed in **Fig. 2**. The dotted and dashed lines mark, respectively, *EF* = 1 and *EF* = 5, the expected limits for (maximum pure) geogenic nature and (minimum mostly) anthropogenic nature. One can see that for Hg and Zn, the geogenic origin is discarded, while for Cr the geogenic origin is the most probable. For Cu, on the other hand, there seems to be a mix of both crustal and anthropogenic sources.
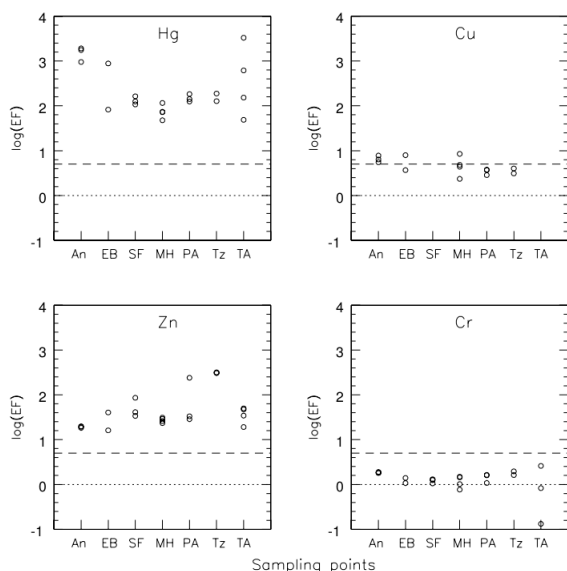

Fig. 2. Atmospheric enrichment factor (*EF*) calculated for Hg, Cu, Zn and Cr from toxic elements concentrations measured in Guanajuato's tunnel network, sections: (An) *Ángeles*, (EB) *El Barretero*, (SF) *Santa Fé*, also known as *Juan Valle*, (MH) *Subterránea Miguel Hidalgo y Costilla*, (PA) *Ponciano Aguilar*, (Tz) *Tamazuca* and (TA) *Tiburcio Álvarez*. The dotted line is the maximum limit estimated for the metals that have only a crustal origin, and the dashed line is the inferior limit for the metals dominated by anthropogenic sources.

To infer the possible anthropogenic metal sources for the indoor air of these tunnels we considered literature information. For instance, Zn and Cu atmospheric input are usually associated to vehicular emissions (e.g., 28 and 29). Zn detected in the PM could came from wear and tear of vulcanized rubber tires, lubricating oil and corrosion of galvanized vehicular parts [30]. Since all these tunnels have a heavy vehicular traffic, the combustion of fossil fuels, such as gasoline and diesel oil, may be considered the main contributors of metals in the indoor air of the tunnels.

The case of Hg is peculiar: we saw above that, although the concentration in the walls is, on average, above the measured values in *T. usneoides* samples, the highest local values in the air correspond only partially to the highest values in the respective tunnels. Hg also presented the highest *EF* values, reinforcing that crustal origin is not the main source. The lack of information about the distribution of this metal in the natural rocks of the region (although a mining zone) hinders a complete analysis of its main anthropogenic sources for the tunnels indoor air. This is an important next step because this metal is very toxic, even at low concentrations. In the case, pedestrians may be continuously exposed to Hg vapor inside the tunnels.

3. Material and Methods

Sampling

In November 2011, plants of *T. usneoides* were collected at Sierra de Santa Rosa, an environmentally preserved area located in the outskirts of Guanajuato town. The plants were washed with distilled water, and dried at room temperature. The younger parts of the plants were selected for transplantation. Then, fifty grams of the plants were introduced in the baskets (**Fig. 3**), each basket was put in Ziploc® plastic bags, individually, wrapped with aluminum foil (to protect from light and prevent contamination), and maintained at 4°C until the installation in the sampling points.
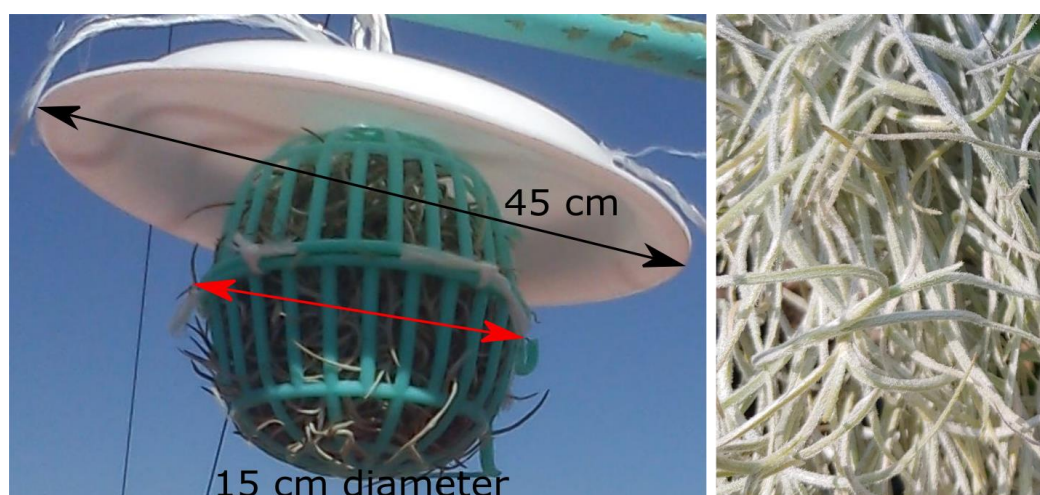

Fig. 3. Bioindicator: (*left*) photo of an installed sampler, and (*right*) photo of the used *Tillandsia usneoides*.

Seven tunnels were selected for the present study (*Ángeles*, *Calle Subterránea*, *El Barretero*, *Ponciano Aguilar*, *Santa Fé*, *Tamazuca* and *Tiburcio Álvarez*), covering a total distance of about 5.4 km long (775m, on average, per tunnel; **Fig. 4**). Two to four baskets were installed by tunnel, positioned at a height between 2.5 to 3 m from the floor, and

when possible, at higher levels. Two additional baskets were reserved for control and were maintained in their Ziploc® plastic bags wrapped in aluminum film, and then stored in the refrigerator again at 4°C. These samples were used to assess the background metal concentration before collection. The installed baskets were exposed for a period of 45 days. After

this period, they were recovered, put in Ziploc® plastic bags, also wrapped in aluminum film, and maintained at 4°C until the analysis.
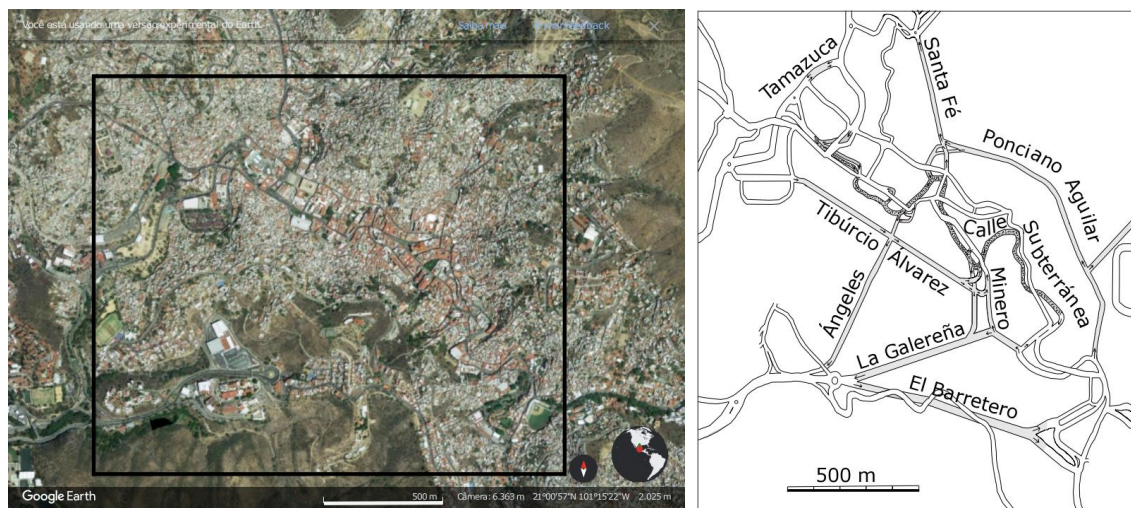

Fig. 4. (*Left*) Map of Guanajuato town center, with a box indicating the zoomed tunnel network map (*right*). The tunnels are painted gray, with the names and directions of the respective sections. The subterranean street Miguel Hidalgo y Costilla is marked in a darker color.

Surface layer scraps of the tunnel walls, close to where the baskets were installed, were collected to prepare a mixed sample for each sampling point (**Fig. 5**). Before sampling, the wall was brushed and then 1 to 2 mm of the wall were scraped off with a plastic spoon, transferred to a conical flask and maintained at 4°C until the analysis. After sifted (1 mm mesh) the metal concentration measurement was performed.

Fig. 5. Details of sampled wall tunnels. The biggest panel shows a general view of one tunnel while the smaller point specific sites where the wall samples were scraped.

Metal analysis

The metal content was determined at the Eduardo Penna Franca Radioisotopes Laboratory (LREPF-IBCCF-UFRJ), Rio de Janeiro, Brazil. During the analysis, blanks were run through to check for any contamination, and the measurements were done in triplicate (3 sample digestions). In order to reduce vapor losses during digestions, 50 cm long glass tubes were used for Hg, while for the other metals we used Teflon® vials covered with watch glass. In both cases, the wall and cover were rinsed, recovering possible residues. A certified analytical grade reference sample (International Atomic Energy Agency certified reference - IAEA) was used for calibration and analytical control quality. As reference material, IAEA-140 (seaweed homogenate, *Fucus sp.*), for *T. usneoides*, and contaminated soil, for tunnel wall scraps, were use. The results obtained from the certified material presented a recovery of 83% for Pb, 90% for Hg, 98% for Cd and 100% for the other metals, that is an average 96% of confidence on the results.

The metals Cu, Zn, Cr, Cd, Fe and Pb were quantified by flame AAS (Varian Spectra AA240FS), while Hg concentrations were measured by atomic absorption spectrophotometry with a Flow Injection Mercury System and autosampler (AS-90), both from Perkin-Elmer®. Detection limits were calculated using the formula: $(3 \pm S_b)/X_b$ where S_b is the standard deviation of 6 measurements of the blank and X_b is the mean of the angular coefficient of the calibration curve [31].

Before metal extraction, first the samples were carefully removed from the baskets, dried at 35°C, and cut into small pieces, with lengths lower than 1 mm each. For the total metal extraction (except Hg) of *T. usneoides* the method described by Vianna *et al.* (2011) [6] was used, briefly: 1 g of the plants was calcined for 2 days at 400°C; after, it was digested with 5 mL concentrated HNO_3 and 1 mL concentrated HCl (Merck® P.A.) on a hot plate at 90°C; after the final solutions were evaporated and suspended to 20 ml HCl 0.1 mol.L⁻¹ and then the metals were quantified. For tunnel wall samples, the method used presented some differences from that used for the plants. First, 0.5 g of sifted samples (thinner particles fraction) were digested for 12 hours at 123°C. After, it was digested with 5 mL of concentrated HF and 4 mL concentrated HNO_3 (Merck® P.A.). Following, all digested samples were transferred to a Teflon® flask and digested with 5 mL HNO_3 until dry on a hot plate, and after with 5 mL concentrated HCl (Merck® P.A.). Finally, the digested samples were filtrated and washed with HCl 0.1 mol.L⁻¹, then it was suspended until 50 mL with HCl 0.1 mol.L⁻¹ and the metals were quantified.

For the Hg analysis, the method suggested by Malm *et al.* (1998) was used [11]. First, 0.1 g of each plant or 1 g of sifted samples of the tunnel walls were digested with 5 mL concentrated HNO_3 and 1 mL H_2O_2 at 100°C (the plants during 1 h and the wall samples during 10 min.). After, 4 mL $KMnO_4$ 10% were added (or 5 mL $KMnO_4$ 5% for the wall samples) and then the samples were digested once more (30 min. for the plants and 10 min. for the wall samples). Thereafter, the samples were digested for 18h at room temperature. The next day, 1.5 mL hydroxylammonium chloride (H_2ONH_3Cl; 12%) was added and the digested samples were re-suspended with Milli-Q® water, and finally, the Hg concentrations were measured [32].

Contaminant level assessment

To infer the possible origin of trace metals, the ratio of atmospheric elementary concentration relative to its concentration in the crust, called enrichment factor (*EF*, **Eq. 2**), must be calculated [33]:

$$EF = \frac{\left(\frac{X}{R}\right)_{air}}{\left(\frac{X}{R}\right)_{crust}}$$

(Eq.1)

where, X is the concentration of the target element and R is the concentration of the reference element. Both for X and R we took the respective measured concentrations and subtracted the respective control ones, that is: $X = X_{meas} - X_0$ and $R = R_{meas} - R_0$, to infer the element retention in *T. usneoides* only during the exposition period. Following Nazir *et al.* (2011) [33], the reference element used here was Fe because this element is entirely of crustal origin, occurs on concentrations substantially higher than that of other elements and is also of low toxicological concern [34]. Crustal mean abundances of studied elements were taken from CRC handbook [35]. It is important to note that the fraction X/R, measured in the same units, is a dimensionless parameter, such that we must guarantee that both X and R are measured in a consistent way. As indicated above, according to Fonseca *et al.* [8] the concentration of Hg in *T. usneoides* is linearly proportional to the concentration of Hg measured in a sophisticated vapor camera, and, assuming the same linear proportionality for the other elements under study (in the lack of similar studies for these elements), we can consider the fraction X/R measured in *T. usneoides* to be representative of this ratio in the indoor air of the tunnels.

The *EF* tells us if the target element is of geogenic nature or if it is enriched by some anthropogenic sources. When the calculated *EF* is close to one, it indicates its crustal origin; between 1 to 5 may have originated from both anthropogenic and crustal sources; and values higher than 5 are considered predominantly to come from anthropogenic emissions (see Jena & Singh 2017 and references therein) [2].

4. Conclusions

The main conclusions of this work are the following:

- Although we can't compare quantitatively the concentrations measured in *T. usneoides* and the ones measured in the walls –plants actively accumulate the toxic elements as opposed to walls– qualitatively the suspended toxic elements in the indoor air of Guanajuato's tunnels revealed to be significantly higher than the concentrations measured in the walls of the same tunnels. This could clearly be seen for Fe, Cr, Cu and Zn.

- The pattern of indoor air and wall crust concentrations for Hg were different: on average, the concentration in the walls is higher, but both low and high values were found in both *T. usneoides* and wall scrap samples. More interestingly, the points with high measured values in the walls usually do not coincide with the points with high measured values in the indoor air.

- Absolute ranges of measured concentrations of suspended toxic elements were in the same order of magnitude of the ones found in the literature for big cities like São Paulo and Mexico City for Fe, Zn, Cu and Cr. The peculiarities found in our samples were: the high values of Zn in *Ponciano Aguilar* and *Tamazuca* tunnels, and the relatively normal concentrations of Cr.

- For the Hg, the indoor air measured concentrations found were also particularly high, especially in the tunnels *Ángeles*, *El Barretero* and *Tiburcio Álvarez*. In addition, Hg occurs in the air mainly in gaseous form (99-95%), which has an additional significance in the active and passive accumulation.

- Concerning the origin of the suspended toxic elements, *EF* estimated for Cr suggest mostly geogenic sources, for Cu a mix of geogenic and anthropogenic ones, while Zn and Hg are clearly most of anthropogenic origin. The main anthropogenic metal sources for the tunnels are probably the vehicular tracks, by direct burning of vehicular fuels or from wear and tear of vulcanized rubber tires. The results point to at least two main environmental problems to be solved: the mercury and zinc sources. Also, it serves to alert the local authorities and the people that visit the Guanajuato's tunnel network that this environment is not contamination free to be used as public routes for pedestrians. Formal air quality evaluations, including composition of suspended particles and gases, must be done to confirm and extend the results of the present work.

Since *T. usneoides* proved to have potential as bioindicator to infer the air quality, being also an easy plant to obtain (abundant and inexpensive), we intend to use this bromeliad to perform a broader study, installing this plant in several other points within the town, in the surroundings and in reference points free of pollution, to verify possible anthropogenic sources of toxic elements.

Supporting Information

Acknowledgments

Author Contributions

EMS Brito, JPM Torres and O Malm designed, conceptualized the research and acquired funding. PA Zarate-Santoyo, JC Valerdi-Negreros, AS Lino and EMS Brito performed the experiments. EMS Brito and CA Caretta performed the data analysis. EMS Brito and PA Zarate-Santoyo conducted most of investigation. EMS Brito and CA Caretta wrote the original draft of manuscript. All authors discussed, read, and approved the submitted manuscript.

References and Notes

[1] Wandke, A.; Martínez, J. *Econ. Geol.* **1928**, *23, 1.* [Crossref]

[2] Jena, S.; Singh, G. *Atmos. Pollut. Res.* **2017,** *8, 490*. [Crossref]

[3] Nriagu, J. O.; Jozef, M. P. *Nature* **1988**, *333, 134*. [Crossref]

[4] Roy, D.; Ahn, S. H.; Lee, T. K.; Seo, Y. C.; Park, J. *Transp. Res. Part D: Transport and Environment* **2020**, *89, 102618*. [Crossref]

[5] Boamponsem, L. K.; de Freitas, C. R.; Williams, D. *Atmospheric Pollution Research* **2017**, 8, 101. [Crossref]

[6] Vianna, N. A.; Gonçalves, D.; Brandão, F.; de Barros, R. P.; Amado Filho, G. M.; Meire, R. O.; Torres, J. P. M.; Malm, O.; Júnior, A. O.; Andrade, L. R. *Environ. Sci. Poll. Res.* **2011**, *18, 416*. [Crossref]

[7] Chiarantini, L.; Rimondi, V.; Benvenuti, M.; Beutel, M. W.; Costagliola, P.; Gonnelli, C.; Lattanzi, P.; Paolieri, M. *Sci. Tot. Environ.* **2016**, 569, 105. [Crossref]

[8] Fonseca, M. F.; Bastos, W. R.; Pinto, F. N.; Rebelo, M. de F.; Torres, J. P. M.; Guimarães, J. R. D.; Pfeiffer, W. C.; Marques, R. G.; Malm, O. *J. Braz. Soc. Ecotoxicol.* **2007**, 2, 129. [Crossref]

[9] Giampaoli, P.; Wannaz, E. D.; Tavares, A. R.; Domingos, M. *Chemosphere* **2016** *149, 14*. [Crossref]

[10] Pereira, M. S.; Heitmann, D.; Reifenhauser, W.; Meire, R. O.; Santos, L. S.; Torres, J. P. M.; Malm, O.; Korner, W. *Chemosphere* **2007**, *67, 1736*. [Crossref]

[11] Malm, O.; Fonseca, M. F.; Miguel, P. H.; Bastos, W. R.; Pinto, F. N. *Sci. Tot. Environ.* **1998**, *213, 57*. [Crossref]

[12] Sutton, K. T.; Cohen, R. A.; Vives, S. P. *Ecol. Indic.* **2014,** *36, 392*. [Crossref]

[13] Sánchez-Chardi, A. *Atmos. Environ.* **2016**, *131, 352*. [Crossref]

[14] Benítez, Á.; Medina, J.; Vásquez, C.; Loaiza, T.; Luzuriaga, Y.; Calva, J. *Diversity* **2019**, *11, 28*. [Crossref]

[15] Zheng, G.; Zhang, R.; Zhou, F.; Li, P. *Int. J. Phytoremediation* **2021**, *23*, 400. [Crossref]

[16] Kim J.J.; Park J.; Jung S.Y.; Lee S.J. *J. Hazard Mater.* **2020** *393*, 122401. [Crossref]

[17] Uzu G.; Sobanska S.; Sarret G.; Munoz M.; Dumat C. *Environ. Sci. Technol.* **2010**, *44*, 1036. [Crossref]

[18] Xiong T.-T.; Leveque T.; Austruy A.; Goix S.; Schreck E.; Dappe V.; Sobanska S.; Foucault Y.; Dumat C. *Environ. Geochem. Health* **2014**, *36*, 897. [Crossref]

[19] Figueiredo, A. M. G.; Nogueira, C. A.; Saiki, M.; Milian, F. M.; Domingos, M. *Environ. Poll.* **2007**, *145, 279*. [Crossref]

[20] Zambrano-García, A.; Medina-Coyotzin, C.; Rojas-Amaro, A.; López-Veneroni, D.; Chang-Martínez, L.; Sosa-Iglesias, G. *Atmos. Chem. Phys*. **2009**, *9, 6479.* [Crossref]

[21] Rodríguez-Castillo, R.; Armienta, M. A. IAHS Publications-Series of Proceedings and Reports-Intern Assoc Hydrological Sciences, 1994, 222, 279.

[22] Piñón-Castillo, H. A.; Brito, E. M. S.; Goñi-Urriza, M.; Guyoneaud, R.; Duran, R.; Nevarez-Moorillon, G. V.; Gutiérrez-Corona, J. F.; Caretta, C. A.; Reyna-López, G. E. *J. Appl. Microbiol.* **2010**, *109, 2173*. [Crossref]

[23] Brito, E. M. S.; Piñón-Castillo, H. A.; Guyoneaud, R.; Caretta, C. A.; Gutiérrez-Corona, J. F.; Duran, R.; Reyna-López, G. E.; Nevárez-Moorillón, V. G.; Fahy, A.; Goñi-Urriza, M. *Appl. Microbiol. Biotechnol.* **2013**, *97, 369*. [Crossref]

[24] Randall R, J. A.; Saldana A, E.; Clark, K. F. *Economic Geology* **1994**, *89*, 1722. [Crossref]

[25] Loria, A.; Ramos-Arroyo, Y. R.; Rocha, D.; Cruz-Jiménez, G.; Razo-Soto, I.; de la Torre, M. C. A.; Armstrong, D.; Guerrero, S.; Wang, F. (2022). *Sci. Tot. Environ.* **2022**, *843, 157093*. [Crossref]

[26] Kim, K. H.; Kabir, E.; Jahan, S. A. *J. Hazard. Mat.* **2016**, *306, 376*. [Crossref]

[27] Amado Filho G.M.; Andrade L.R.; Farina M.; Malm O. *Atmos Environ*. **2002**, *36*, 881. [Crossref]

[28] Alolayan, M. A.; Brown, K. W.; Evans, J. S.; Bouhamra, W. S.; Koutrakis, P. *Sci. Tot. Environ.* **2013**, *448, 14*. [Crossref]

[29] Basha, A. M.; Yasovardhan, N.; Satyanarayana, S. V.; Reddy, G. V. S.; Kumar, A. V. *Atmos. Poll. Res.* **2014**, *5, 591*. [Crossref]

[30] Karar, K.; Gupta, A. K.; Kumar, A.; Biswas, A. K. *Environ. Monit. Assess.* **2006**, *120, 347*. [Crossref]

[31] Silva, A. P.; Alves, M. C. C. Como iniciar a validação de métodos analíticos. Congresso e Feira da Qualidade em Metrologia Rede Metrológica do Estado de São Paulo, São Paulo, Brazil. May 30 - June 01, 2006.

[32] Bastos, W. R.; Fonseca, M. F.; Pinto, F. N.; Rebelo, M. F.; Santos, S. S.; Silvera, E. G.; Torres, J. P. M; Malm, O.; Pfeiffer, W. C. M. *Environ. Res.* **2004**, *96, 235*. [Crossref]

[33] Nazir, R.; Shaheen, N.; Shah, M. H. *Atmos. Res.* **2011**, *101, 765*. [Crossref]

[34] Wedepohl, K.H. *Geochim. Cosmoch. Acta* **1995**, *59, 1217*. [Crossref]

[35] Lide, D.R. CRC handbook of chemistry and physics, 2004, Vol. 85. CRC Press.

How to cite this article