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TILLANDSIA USNEOIDES AS BIOMONITOR OF AIR POLLUTION

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Abstract

T. usneoides is one of the most employed organisms for biomonitoring atmospheric metals due to its ability to accumulate and retain pollutants, including trace elements. In this article, we review the use of *T. usneoides* as biomonitor of atmospheric metals, as well as the different types of biomonitoring, sample preparation methodologies and analytical techniques used in the studies performed in the last two decades. We also present the most important results of *T. usneoides* that have been obtained in different biomonitoring studies and geographical areas around the world. According to the results of these studies, *T. usneoides* is a sensitive and selective biomonitor for some metals, able to respond quickly to small changes in atmospheric composition, making it a suitable option for comparing sites or for biomonitoring short-term events. However, it is necessary to validate its implementation with calibration curves made in the environment under study. In summary, there is an important opportunity area for the study of physiological parameters in *T. usneoides* as response of pollutants, which could be useful as biomarkers of exposure and effect.

Key words: metals, trichomes, biomonitoring, bioaccumulation, Bromeliaceae family

1.0 Introduction

Tillandsia is a genus that belongs to the Bromeliaceae family, which has been widely used as biomarker and biomonitor of air pollution. These epiphytic plants have proven to be a suitable biomarker of exposure in the presence of some pollutants (Wannaz and Pignata, 2006), since they are able to reflect in their tissues atmospheric levels of some toxic elements. Among the most commonly used species for air monitoring we found *T. usneoides*, *capillaris* and *recurvata*. Other less-used species are *T. tricholepis*, *permutata*, *caput-medusae morren*, *fasciculata*, *brachycaulos*, *pruinosa*, *velutina*, *albida*, *balbisiana*, *paucifolia* and *utriculata* (Brighigna *et al*., 1997; Bermudez et al., 2009, Techato *et al*., 2014; Kovácik *et al*., 2014).

Tillandsias have specialized structures on leaves, called trichomes, which allow the absorption of moisture and nutrients available in the atmosphere. These structures also make possible the absorption of atmospheric pollutants (Elías *et al*., 2008; Pellegrini *et al*., 2014). These plants have a complex metabolic adaptation known as crassulacean acid metabolism (CAM) that reduces the possibility of drought stress (Haslam *et al*., 2003), enhancing their survival or their easy adaptation to other ambient conditions. They are widely distributed in the deserts, forests and mountains of Central America, South America, Mexico and the southern United States of America (Garth, 1964).

Due to the ability shown by *T. usneoides* to act as biomonitor of air pollutants, it has been used to evaluate the air quality from different sources such as metal-mechanical industries, chemical and metallurgy, cement, vehicular pollution as well as unpolluted areas. An important advantage of using the technique of biomonitoring of air pollutants is the information that these living organisms can provide, such as the physiological effects of pollution. These are also called biomarkers, and constitute a real answer of biota to industrial emissions, giving a first-level evaluation of the health of an ecosystem.

T. usneoides has been used as biomonitor for atmospheric metals, polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and nuclides monitoring (Li *et al*., 2012; Martínez-Carrillo *et al*., 2010, de Souza Pereira *et al*., 2007). Due to the wide distribution of *T. usneoides* in the American continent and their ability to adapt to different environmental conditions, it has been used as an active and passive biomonitor, with several exposure times, and different conditions of sampling and sample preparation.

In this article, we review the use of *T. usneoides* as biomonitor of atmospheric metals, as well as the types of biomonitoring, sample preparation methodologies and analytical techniques used in the studies reported in the last two decades. We also present the most important results of *T. usneoides* that have been obtained in the surveys performed in different geographical areas around the world.

2.0 Atmospheric biomonitoring

Biomonitoring is defined as the use of organisms for obtaining information about certain characteristics of the biosphere (Marć *et al*., 2015). Hence, biomonitors are organisms that provide quantitative information about the environment in which they develop. This implies that the biomonitor should concentrate the contaminant of concern and quantitatively reflect their environmental conditions (Wolterbeek, 2002). Therefore, biomonitors provide information about the concentrations of pollutants and the different effects that these have on living organisms (Market, 2007).

In contrast to conventional physical and chemical methods, biomonitoring allows potential toxic air pollutants to be assessed directly, by providing biological information that may be used to estimate the environmental impact of specific pollutants on other organisms including humans (Carreras *et al*., 2009). So, biomonitoring is a competitive analytical alternative, which involves the continuous survey of an area (Kettrup and Marth, 1998; De Temmerman *et al*., 2004). Biomonitors might be very convenient because of their high sensitivity to a broad spectrum of substances and to their tolerance to high levels of substances accumulated in the tissues over a period of time.

The biomonitoring technique is fast and economical, and allows a high number of observation units (as well as samples and sampling sites) to be employed. Furthermore, due to the low maintenance of these organisms, they can be used to monitor large areas. Also, by observing the different responses of biomarkers that can be developed from exposure to contaminants, the effect of pollutants in living organisms and ecosystems can be made clear (De Temmerman *et al*., 2004; Rubiano and Chaparro, 2006). With the use of large scale biomonitoring, it is possible to identify contaminated areas, to make distribution maps of contaminants and to elucidate their transport routes. By applying appropriate statistical tools, pollution sources can be detected and classified. Moreover, storage banks for selected samples might be implemented.

Various organisms have been used to monitor atmospheric pollutants such as lichens, mosses, ferns, grasses, bark and tree rings, and pine needles. For all the biomonitors used, the mechanism of absorption and retention of trace elements is still poorly documented (Szczepaniak and Biziuk 2003). Conti and Cecchetti (2001) indicated that the accumulation of elements by plants is influenced by factors such as the availability of the element and its properties, as well as by characteristics intrinsic to the plant, such as the species, age, health status and type of reproduction. Finally, environmental factors as temperature, moisture availability, and soil characteristics, among others, also modify the extent of chemical accumulation in a biomonitor.

2.1 Criteria for selecting organisms in atmospheric biomonitoring

A main aspect to take into account is the cumulative behavior of the pollutants in the biomonitor through time (Wolterbeek, 2002). The chosen organism must be tolerant to the target atmospheric pollutants and to a wide range of concentrations. Besides, it is important that the organism is endemic to the study site, because it must be available all year round for sampling purposes.

In addition to the aforementioned criteria, the biomonitor should have the following characteristics:

- The organism should be easily recognizable and collectible (Namieśnik and Wardencki, 2002).
- It should have a low initial content of the pollutant of interest.
- The concentrations of the pollutant in the biomonitor should be evaluated by routinary techniques.
- The sampling methods, as well as the procedures of sample preparation must be as simple and fast as possible.
- The biomonitor must reflect quantitatively the environmental conditions prevailing during the exposition.
- The physiological mechanisms underlying the accumulation of atmospheric pollutants in the biomonitor should be known, in order to facilitate the interpretation of the results.
- The organism must be sensitive to changes in the concentrations of the pollutants present in the study site (*i. e.*, to show a clear dose-effect relationship). In this way,

any significant change in the biomonitor behavior or in its physiological parameters (biomarkers) should reflect the availability of the pollutants of interest.

2.2 Types of biomonitoring

According to Market (2007), the biomonitors can be classified as passive or active according to their origin. The passive biomonitors are organisms found naturally in the study area. These biomonitors allow to know the cumulative concentration of a pollutant, as well as the effects that it produced in the long term. The active biomonitors are organisms taken from elsewhere and transplanted to the study site, where they stay for a specific period of time and under certain conditions (Ĉeburnis and Valiulis, 1999). These organisms should come from uncontaminated areas; however, nowadays it is difficult to find sites that have not been impacted by human activities or natural sources that increase the concentrations of trace elements in the biomonitor. This kind of biomonitoring is useful to know the time and type of response of the biomonitor to changes in its environment (accumulation, adaptation, etc.) The effects and responses of active biomonitors can be studied after a certain time in order to compare them to those presented by organisms placed in a control site. To evaluate the effects of air pollutants, it is also important to assess in the biomonitor the concentration of the element, or other physiological parameters, before and after the exposure period.

2.3 Sample size

A biomonitoring method, either passive or active, is valid statistically only if samples analyzed are representative of the study area. Accordingly, several simple random samples must be collected from the same site in order to represent the local variability of the elements analyzed. The minimal number of samples (*n*) recommended to carry out a passive biomonitoring is between 5 and 10, which must be collected from a surface of about 50 m x 50 m, independently of the species used as biomonitor, the pollution level or the latitude of the sampling site (Rühling, 1994). Fernández *et al*. (2002) demonstrated that, for a given pollutant, it is necessary to gather at least 30 samples, in order to obtain an error of about 20% in the mean determination. Nonetheless, this error value is higher than that is commonly recommended for a pollution survey study.

As there is scarce information about the sample size in biomonitoring studies, Aboal *et al.* (2006) and Varela *et al.* (2010) have proposed a method to calculate the number of samples necessary to distinguish between several sites on the basis of the concentrations measured in mosses. Aboal *et al*. (2006) found that to differentiate between an uncontaminated sampling area and another slightly contaminated one, 30 samples are required from each site.

2.4 Preparation of the sample. Active and passive biomonitoring

The aim of this operation is to obtain biological material as homogeneous as possible. In the case of active biomonitoring, the material to be transplanted (and coming from an unpolluted site) is first selected by discarding the dehydrated, non-viable parts of the plant and keeping only the alive ones, which can be distinguished easily by their bright green color. Afterwards, the plant is washed to remove the particle material from its surface. One or several washings can be carried out, most frequently from one to three washings with slightly acid distilled water. The plant can be dried at room temperature and then transplanted in the study site.

In relation to passive biomonitoring, the biological material is selected *in situ*. It is important to sample enough plant mass (25 to 250 g of plant tissue) to have a sufficient quantity of alive specimens prior the analysis. It is also important that the samples keep all the material deposited at their surface until their analysis. For this, it is suggested that the biomonitor be transported in individual bags before being analyzed. The biomonitor must not be washed before its chemical analysis.

2.5 Duration of the exposure

Biomonitoring techniques are not standardized yet, and therefore the exposure time is commonly established according to the specific needs of the study. Ratcliffe (1975) proposed several criteria to define the most adequate exposure time if the accumulation of pollutants has to be evaluated: i) the levels of the pollutants accumulated in the biomonitor should be measurable; ii) the values found should be reliable and reproducible in an acceptable range of concentrations of the target pollutant, and iii) the exposure time should comply with the practical requirements of the study.

In some studies, biomonitors such as *T. usneoides* have been exposed from 5 to 90 days to the atmospheric pollution of a specific area, while several authors have conducted seasonal studies that discriminate the exposure during periods of rain and drought (Martínez-Carrillo *et al.*, 2010; Isaac-Olive *et al.*, 2012). Ares *et al*. (2012) showed that the accumulation of pollutants a in biomonitoring study exposing mosses during 12 weeks is greater than the accumulation of pollutants occurred in two consecutive periods of six weeks each. The authors concluded that, in that study area, the exposure time (6 or 12 weeks) did not bring about any saturation of the capacity for bioaccumulating pollutants in the moss.

By considering these results, we propose exposure periods from 30 to 45 days. These periods are long enough to guarantee both the accumulation of pollutants and the reproducibility of the results. Actually, this exposure period is the most frequently reported in the bibliography. Besides, if there is a linear relationship between the pollutant accumulation and the time of exposure, the slope of the regression is generally more pronounced during this period.

Although it is expected that the pollutant concentrations in the biomonitors increase linearly during the exposure time, this has been observed only for some chemical elements that are either present at high levels in the air or non-essential to the development and physiological activities of the organisms.

2.6 Methods for trace elements analysis in biomonitors

The studies carried out to assess trace elements in a biomonitor generally involve as first step an acid digestion followed by analytical techniques such as atomic absorption spectroscopy (AAS), laser ablation (LA), inductively coupled plasma (ICP) spectroscopy, mass spectroscopy (MS), particle-induced X-ray emission (PIXE) spectrometry, and neutron activation analysis (NAA) (Bi *et al.*, 2001; Figueiredo *et al.* 2001; Martínez-Carrillo *et al.*, 2010; Isaac-Olive *et al.*, 2012). Some plant specimens such as those of *T. usneoides* are hard to digest (Bi *et al.*, 2001), and so this step must be optimized in each case to obtain reliable results.

3.0 *Tillandsia usneoides* as biomonitor of air pollution

3.1. Physiological characteristics of *Tillandsia usneoides* L.

T. usneoides L. is widely distributed in the Americas; it can be found from southern USA to Argentine (Garth, 1964). This photosynthetic, non-parasitic species is classed as epiphyte because it lacks of roots, grows in absence of soil and gets all essential nutrients and water from the air. The plant consists of pendant stems even longer than 6 meters and produces small but fragrant flowers, probably by self-pollination (Hansford *et al.*, 1973). New individuals can originate from dispersing seeds or by vegetative propagation.

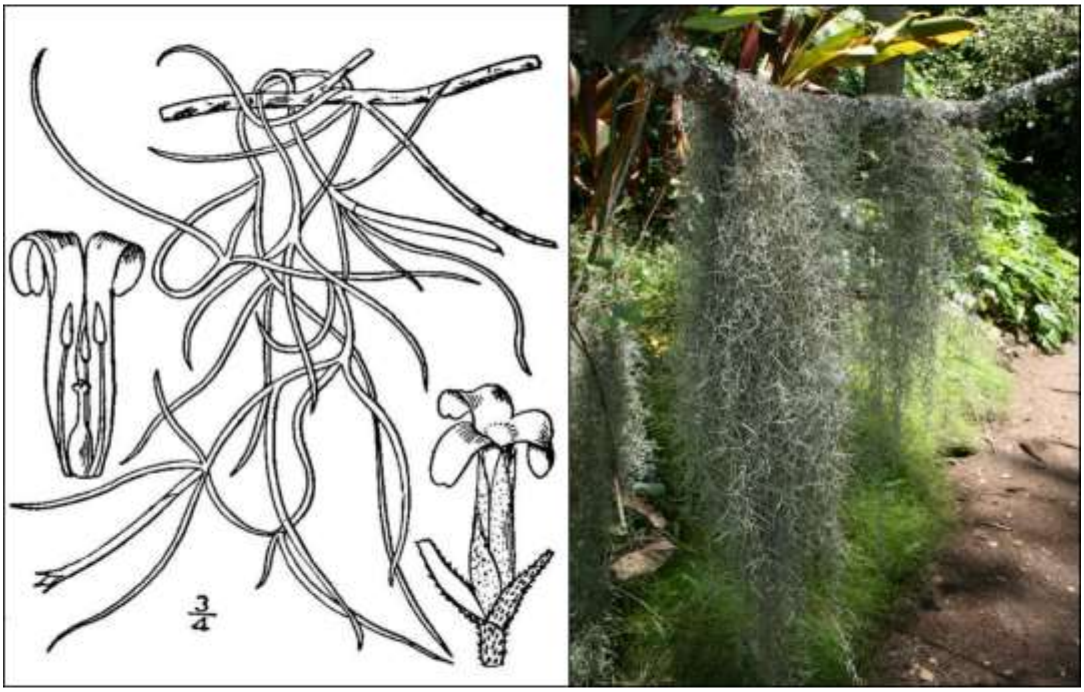

Figure 1. Left: Morphological features of *Tillandsia usneoi*des. Right: Plant growing down from the branch of a tree. Available in http://tropical.theferns.info/image.php?id=Tillandsia+usneoides

T. usneoides has curved and filiform leaves, of about 2-7 cm long and 1-2 mm width (Figure 1, left), which are covered by a dense trichoma layer that produces a silver-gray appearance (Thomas y Lineham, 1986). *T. usneoides* lacks of functional xylem, and has their water absorption organs (*i. e*., trichomes, which are specialized, clove-shaped structures) distributed in almost all its surface, excepting in some of the flowering organs. If the plant is dry, the leaves are gray due to the air enclosed in the trichomes, but if it is wet, the air is substituted by water and the plant turns dark green (Billings, 1904; Wagner *et al*., 2004). Papini *et al.* (2010) indicate that *Tillandsia* spp. use their epidermal trichomes for absorbing atmospheric water, as well as mineral and organic nutrients. These structures increase the surface area exposed to the air and hence the ability of the plant to absorb water and to withstand under drought conditions.

3.2. Mechanisms of atmospheric metal accumulation in *Tillandsia usneoides*

T. usneoides is one of the most employed organisms for biomonitoring atmospheric metals due to its ability to accumulate and retain pollutants, including trace elements. This plant has a very rustic root system and their trichomes constitute the only route by which chemicals are drawn (*i. e.*, from the surrounding atmosphere; Korzekwa *et al*., 2007). In addition to nutrient absorption, the trichomes of *T. usneoides* perform the following functions:

- Protection against solar radiation by increasing the reflecting surface,
- Mechanical block of mineral nutrients and organic particles, which can be later transported inside the leaves,
- Protection of the plant by reducing the evapotranspiration of water (Papini *et al*., 2010). This mechanism leads to the closing of the stomata during the day, thereby avoiding excessive water losses at high temperatures (Malm *et al*., 1998).

The deposition of pollutants or nutrients in *T. usneoides* can occur by the following mechanisms: a) wet deposition (*i. e.*, directly by means of rain); b) dry deposition of dust transported by the wind; and c) deposition of waste originated from the decay of the plant acting as support of *T. usneoides*.

4.0 Biomonitoring of inorganic pollutants using *Tillandsia usneoides*

One of the most widespred uses of *T. usneoides* has been the biomonitoring of metals. Among the elements that have been shown to accumulate in this species we found Al, As, Ba, Br, Ce, Cl, Co, Cu, Fe, Hg, K, La, Mg, Sm, Ti, Th, V, and Zn (Figueiredo *et al*., 2001, 2007; Martínez-Carrillo *et al*., 2010; Isaac-Olivé *et al*., 2012; Pellegrini *et al*., 2014; Table 1). Some authors, such as Calvario-Rivera (2012), have determined how the plant tissues reflect the atmospheric levels of Ca, Cd, Cu, Fe, K, Mn, Na, Ni, Pb, Sr and Zn. Moreover, the relationship between the accumulation of these metals and the production of MDA (*i. e.*, malondialdehyde, a biomarker of oxidative stress) and chlorophylls has also been explored in this biomonitor (Calvario-Rivera, 2012).

Due to the toxicity of mercury and the profusion of sites polluted with this metal, several studies have focused on its accumulation in *T. usneoides*. Calasans and Malm (1997) carried out an active biomonitoring in a chlor-alkali plant, and they concluded that *T. usneoides* constitutes an efficient biomonitor of atmospheric Hg, even under the stressing conditions prevailing in this kind of industries (as the high temperatures, elevated Hg concentrations and the oxidizing environment). According to these authors, a period of 15 days is optimum for monitoring atmospheric Hg with *T. usneoides* (Calasans and Malm, 1997; Table 1). After that, Malm *et al*. (1998) demonstrated the usefulness of this organism for an active biomonitoring at full scale in the city of Alta Floresta, Brazil. In this study, the spatial (with respect to known emitting sources) and seasonal (rain and drought periods) variations of atmospheric Hg were evaluated (Table 1). The results indicated that the goldshops were critical sources, where the concentration could be up to 12-fold higher than in the surroundings. They have found that, inside these goldshops and in the drought season, the content of Hg in the air could be three times higher than the level measured during the rainy period due to the diminution in the gold production in this period. Filho *et al*. (2002) showed that the scales, stems and leaves of *T. usneoides* provide an abundant contact surface where Hg can be strongly adsorbed. In contrast, in its epidermal cells, only a slight adsorption was observed (Table 1).

Bastos *et al*. (2004) evaluated the performance of *T. usneoides* as biomonitor of Hg in the interior and the exterior of goldshops. They measured the retention rate of Hg under laboratory conditions too. Their results demonstrated that *T. usneoides* respond quickly to the variations of atmospheric Hg, and so can be used for comparing the levels of this metal in inner and external spaces. However, they concluded that it is mandatory to establish calibration curves of the biomonitor in the environment in which it will be exposed, since the humidity and the content of particulate matter might increase the deposition and alter the plant ability to reflect the chemical composition of its environment. This study also indicated that a laboratory experiment cannot truly reproduce the dynamics of the environmental conditions nor the effects of a mix of pollutants, since their interactions affect the answer of the biomonitor (Table 1).

On the other hand, Sutton *et al*. (2014) demonstrated that *T. usneoides* gives a rapid answer to the presence of atmospheric Hg and in a wide range of concentrations of this metal. Moreover, the biomonitor reflect the concentration of Hg even 15 days after the end of the exposition. These features point out that *T. usneoides* is a well-suited biomonitor for surveying changes in the atmospheric content of Hg occurred in short periods (*i. e*., some weeks), as well as for comparing the contents of this metal measured in different sites (Table 1).

Scarce studies have been conducted to evaluate the levels of rare earth metals with *T. usneoides*. Issac-Olivé *et al*. (2012) have studied the relationship between the contents of La, Cs and Sm in air monitoring filters and in the tissues of *T. usneoides* in the industrial zone of Tula Tepeji, Hidalgo, Mexico (Table 1). Lanthanides can be found in industrialized zones associated to the emissions of petroleum refineries that utilize zeolitic additives in the fluid catalytic cracking process (Moreno *et al*., 2008). Although lanthanides are present at very low concentrations in the atmosphere, they can be used as fingerprints of this kind of pollution sources. Issac-Olivé *et al*. (2012) revealed a significant correlation between the content of lanthanides measured in air monitoring filters and in *T. usneoides*. Nonetheless, Figuereido *et al*. (2001) found extremely low contents of La, Ce, Nd, Sm, Eu, Tb, and Yb in *T. usneoides* exposed in three sites of São Paulo, Brazil. These findings could support that these metals are not significantly present in the environmental air of São Paulo city.

In a laboratory study, Li *et al*. (2012) evaluated the potential of *T. usneoides* to monitor ^{133}Cs by exposing the plant to several concentrations of this element (0 a 100 mmol/L). The results evidenced that the content of Cs in *T. usneoides* augmented significantly along with the Cs concentration in the solutions, which suggests that the biomonitor could accumulate this element in a fast and effective way (Table 1).

Martínez-Carrillo *et al*. (2010) exposed *T. usneoides* in four sites of the critical zone of Tula, Mexico, during 10 weeks, to estimate the correspondence between several elements (S, Ca, Si, Ti , Fe, V, Ni, Cu y Zn) accumulated in the biomonitor and in material sampled by air monitoring units. For the analysis, the PIXE technique was used (Table 1). The results showed that *T. usneoides* reached the maximal levels of the elements between the weeks 6-10, and that Ca, S and V were accumulated significantly by the plant. Besides, the PIXE analysis could detect the same number of elements in the biomonitor and in the filters used in the air monitoring units. The authors reported several relationships between the contents of S and V found in the filters and in the biomonitor, as well as low accumulation rates of Fe, Cu and Zn in both monitoring techniques. They concluded that *T. usneoides* is able to differentiate the elements being present at the highest concentrations in the site and the impacts of the most important pollution sources of the studied zone, such as a petroleum refinery and an electric power generation plant. Finally, they found that the active monitoring methodology implemented in the study confirmed to be adequate to assess the impact of the numerous pollution sources of this critical zone.

Calvario-Rivera (2012) studied the effect of Ca, Cd, Cu, Fe, K, Mn, Na, Ni, Pb, Sr, and Zn in physiological parameters of *T. usneoides* such as chlorophylls (*a*, *b* and total) and MDA after the exposure of the biomonitor in four sites of the Tula-Tepeji region in Hidalgo, Mexico (Atitalaquia, Atotonilco de Tula, Tlaxcoapan and Tula de Allende). The biomonitoring was carried out during the drought (from February to April) and the rain (from July to October) seasons of 2008. *T. usneoides* showed a higher affinity for Ca and Pb throughout the drought period and for Cu, Fe and Zn during the rains (Table 1). The maximal accumulation of Pb in the biomonitor was observed during the drought period in Tlaxcoapan, which is considered the receptor site of the atmospheric pollution of the zone (Ortiz *et al*., 2011; Martínez-Carrillo *et al*., 2010), while in the rainy period the maximal accumulation occurred in Atitalaquia and Tula de Allende. The determination of the enrichment factors in both the biomonitor and the filters of the air monitoring units indicated that *T. usneoides* reflect the Pb concentration in the environmental air. Furthermore, the statistical analysis of the results allowed knowing that the metal accumulation in the biomonitor increased its MDA content and has a negative effect on the concentration of photosynthetic pigments and on the deassimilation of Zn and Ni. The authors suggested that *T. usneoides* is a good option to carry out the active biomonitoring of pollutants as it can reflect the atmospheric composition in terms of Ca, Pb, Cu, Fe and Zn.

Other studies have been focused on determining which atmospheric pollutants are retained preferently by *T. usneoides* and the sources of pollution they are associated to. Figuereido *et al*. (2001) performed an active biomonitoring to evaluate the ability of *T. usneoides* for reflecting the fluctuations of the atmospheric levels of 27 elements (Al, As, Ba, Br, Cl, Co, Cr, Fe, K, Mg, Mn, Mo, Na, Rb, Sb, Sc, Ti, Th, V, Zn, La, Ce, Nd, Sm, Eu, Tb, and Yb) in São Paulo, Brazil (Table 1). After eight weeks of exposure, the biomonitor presented higher concentrations of Al, As, Cr, Fe, Mo, Sb, Ti, V and Zn in the most polluted sites; this was interpreted as a good indicator of the plant ability for biomonitoring the air quality in terms of these pollutants. In a later survey, Figuereido *et al*. (2007) observed seasonal variations in the amount of metals accumulated in *T. usneoides*, which were associated to specific pollution sources in São Paulo, Brazil (Table 1). In this way, Zn, Ba, Ca and Sb were associated to vehicle emissions; the elevated concentrations of Co measured in the biomonitor were found in a metal processing plant, while Fe and Rb were rather related to soil particles. Finally, the maximum concentrations were measured in winter, and this was in agreement with the high PM_{10} values determined in other studies at the same season.

In a research conducted by Vianna *et al*. (2011), the accumulations of Cd, Cr, Cu, Pb and Zn by *T. usneoides* in two zones of Brazil (Rio de Janeiro and Salvador) were compared (Table 1). In both cities, the *T. usneoides* composition showed abundances with the following order: Zn > Cu > Pb > Cr > Cd. Significant differences with respect to the control site were also found for all the elements analyzed. Besides, in both cities, the metal concentrations were slightly superior in winter than in summer. This evidenced the capability of *T. usneoides* for comparing relatively similar sites (in terms of pollution level) and for reflecting small changes in the chemical composition of environmental air. Vianna *et al*. (2011) also found that 80% of the particles retained by the biomonitor had a diameter inferior than 10 μm (which correspond to the inhalable fraction of air particulate matter) and that the contents of Zn, Cr, and Cu in these particles might contribute to increase their toxicity. From these findings, the authors concluded that *T. usneoides* constitutes a well-suited biomonitor of heavy metals associated to the inhalable atmospheric particles.

Pellegrini *et al*. (2014) performed an active biomonitoring in Pisa, Italy, to evaluate the ability of *T. usneoides* for accumulating atmospheric pollutants (Al, As, B, Ba, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sb, Sr, V and Zn, Table 1) in sites with different land uses, anthropogenic activities and distances from the emitting sources. According to their results, the high concentrations of Al, Ba, Bi, Cd, Co, Cu, Pb, Sb and Zn were due to the intense vehicular traffic of certain zones. In contrast, the considerable concentrations of Mg, Sr and Zn were associated to industrial sources, whilst in rural sites the main accumulated elements were Fe, Mn, Na, and V. As in previous studies, the data revealed the high resistance of *T. usneoides* to the heavy metal toxicity, as well as its ability to reflect the differences between exposure sites and hence to relate the pollutants with their sources.

Husk *et al*. (2004) have compared the contents of Ca, Cu, Fe, K, Mg, Mn, and Zn in samples of *T. usneoides* collected and analyzed in 1973/1974 and 1998, and they concluded that the concentrations of Ca, Mg K, Cu and Fe varied significantly through time in the biomonitor (Table 1). The first four elements diminished while Fe augmented. The observed decline in the Ca, Mg, K and Cu levels concurred with a global decrease in these elements, reported by Gough *et al*. (1994; cited in Husk *et al*., 2004). Given the high diversity of factors involved, however, the authors could not explain the increase of the Fe concentration nor validate the usefulness of *T. usneoides* to reflect the temporal variability of the atmospheric composition in the studied period.

5.0 Conclusions and perspectives

T. usneoides is a suitable biomonitor for screening studies concerning the metal atmospheric pollution and for identifying possible emitting sources. This biomonitor presents seasonal trends of metal accumulation, similarly to those shown by the conventional air monitoring systems. According to this review, several sample weights, number of samples and exposure times have been employed in the studies conducted during the last 20 years. Therefore, as for any bioassay, it is imperative to standardize the methodologies of active and passive biomonitoring in order to enhance the reliability and reproducibility of the results.

Even though *T. usneoides* has the capability for accumulating pollutants in its tissues, a better understanding of the mechanisms involved in this accumulation is necessary if the use of this biomonitoring technique is to be more widespread. Moreover, it has been demonstrated that the metals bioaccumulated in *T. usneoides* bring about effects on physiological parameters such as the contents of chlorophylls and MDA. Consequently, *T. usneoides* has the potential to be also employed as a biomarker of exposure and effect. To this end, further studies must be performed with other physiological parameters to support and to validate the reliability and versatility of this biomonitor.

Confict of interest: The authors declare that there is no conflict of interest.

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Table 1. Summary of results of biomonitoring of metals using *Tillandsia usneoides*.

Contaminants	**Location**	**Year**	**Sample mass used (g)**	**Exposition time**	**Type biomonitoring**	**Sample preparation**	**Analysis method**	**Changes or concentrations detected**	**Ref.**
Hg	Urban area of Honorio Gurgel, Rio de Janeiro. Brazil	1997	5 g	38 and 68 days	Active	Some samples were washed and others unwashed, before the acid digestion.	Cold vapor - atomic absorption spectrophotometry (AAS)	1 to 10 400 mg kg^{-1}	Calasans and Malm, 1997
Hg	Alta Floresta city and Río de Janeiro, Brazil	1998	5 g	15 and 45 days	Active	The samples were washed with milli Q water, before the acid digestion.	Cold vapor - AAS	26 mg kg^{-1}	Malm et al.
Al, As, Ba, Br, Cl, Co, Cr, Fe, K, Mg Mn, Mo, Na, Rb, Sb, Sc, Ti, Th, V, Zn, La, Ce, Nd, Sm, Eu, Tb and Yb	São Paulo, Brazil	2001	5 g	8 weeks	Active	Acid digestion, unwashed samples	Neutron activation analysis (NAA)	Elements with tendency to increase (mg/kg): Al: 3606 As: 0.65 Cr: 6.7 Fe: 3960 Mo: 1.4 Sb: 1.83 Ti: 373 V : 9.4 Zn: 190	Figueiredo et al. 2001
Hg	Laboratory experiment	2002	5 g	15 days	Active, laboratory study	Acid digestion, unwashed samples	AAS	2702 mg/kg	Filho et al. 2002
Hg	Laboratory experiment and field study in Porto Velho	2004	5g of T.	15, 30, 45 and/or 60 days.	Active: Laboratory and in ex-gold trade shops (closed, restored and	Acid digestion, unwashed samples	cold vapor atomic- AAS	In laboratory chamber 271 – 485 ug/m^3 In Porto Velho, Gold-trade shop 1.15 mg/kg in 15 d	Bastos et al. 2004

Contaminants	Location	Year	Sample mass used (g)	Exposition time	Type biomonitoring	Sample preparation	Analysis method	Changes or concentrations detected	Ref.
	and Alta Floresta, Brazil				nonrestored).			3.22 mg/kg in 60 d In Alta Floresta, closed ex-gold trade shop: 1.42 mg/kg in 15 d 2.11 mg/kg in 45 d	
Ca, Cu, Fe, K, Mg, Mn and Zn	Central Florida, USA	2004	50 g	Samples collected and analyzed in 1973 y 1974 and 1998	Passive	Acid digestion, unwashed samples	Atomic absorption spectrophotometry (AAS, Ca, Fe, K, Mg, Mn and Zn) Graphite furnance-AAS (Cu)	1973/1974 – 1998 (mg/kg) Ca: 4650 – 4160 Mg: 2070 – 1360 Fe: 292.3 – 574.6 Mn: 136.8 – 52.8 Zn: 65.4 – 73.5 Cu: 20.7 – 7.1 K: 5040 - 2550	Husk et al., 2004
As, Ba, Br, Ca, Ce, Co, Cr, Eu, Fe, K, La, Na, Nd, Rb, Sc, Sm, Th, U, Yb and Zn	São Paulo, Santo André, São Caetano and Mauá, Brazil	2007	5 g	5 experiments of 8 weeks each	Active	Freeze-dried, unwashed samples	Neutron activation analysis	Metals significantly differents (mg/kg): Zn: 189 Ba: 51 Fe: 1324 Rb: 48	Figueiredo et al., 2007
S, K, Ca, V, Mn, Fe, Cu, Zn and Sr	Hidalgo, Mexico	2010	25 g	10 weeks	Active	Acid digestion, unwashed samples	PIXE	Elements with higher RAF* (mg/kg) Ca: 26 400 S: 5600 V: 23	Martínez-Carrillo et al., 2010
Cd, Cr, Cu, Pb	Rio de	2011	DNR	45 days	Active	Acid digestion,	ASS	(mg/kg)	Vianna et al. 2011

Contaminants	Location	Year	Sample mass used (g)	Exposition time	Type biomonitoring	Sample preparation	Analysis method	Changes or concentrations detected	Ref.
and Zn	Janeiro, Brazil					unwashed samples		Cd – 0.8 Cr – 4.4 Pb – 11.2 Cu – 13.6 Zn – 385	
Ca, Cd, Cu, Fe, K, Mn, Na, Ni, Pb, Sr and Zn	Hidalgo, Mexico	2012	25 g	2 experiments of 12 week each, dry and rainy season	Active	Acid digestion, unwashed samples	AAS	Seasons: Dry – Rainy (mg/kg) Ca: 20 922 – 9389 Cd: ND – ND Cu: 10.03 – 11.06 Fe:1 040.04 – 1584 K: 7515 – 7370 Mn: 69.68 – 86.12 Na: 9469 – 5975 Ni:2 9.98 – 4.64 Pb: 51.15 – 24.60 Sr: 184.47 – 63.17 Zn: 49.34 – 66.48	Calvario-Rivera, 2012
Ce, La and Sm	Hidalgo, Mexico	2012	200 mg	13 weeks	Active	Freeze-dried, unwashed samples	NAA	(mg/kg) Ce – 4 La – 1.75 Sm – 0.28	Isaac-Olivé et al., 2012
^{133}Cs	Laboratory experiment	2012	3 g	11 days	Active: Each plant was soaked in a cup of 500 mL CsCl water solution for 2 min. at the same time	Samples were washed with bi-distilled water, dried at 70°C for 10 h, ground and reduced to ashes, before acid the	ASS	Biomonitor – Solution mg Cs/kg - mM Cs 0.58 – 0.1 1.34 – 1.0 12.4 – 10.0 54 – 54.0	Li et al., 2012

Contaminants	Location	Year	Sample mass used (g)	Exposition time	Type biomonitoring	Sample preparation	Analysis method	Changes or concentrations detected	Ref.
					every day.	acid digestion.			
Hg	Southeastern Georgia and Northern Florida. In the vicinity of the LCP Chemicals EPA Superfund site in Brunswick, GA	2014	200 g for retention Hg experiment 2.5 g to analyze Hg initial content 5 g for active biomonitoring	Retention Hg: 2, 7 and 14 days Active biomonitoring : 2 weeks	Active	Samples were placed in HNO_3 solution immediately after collected.	Inductively-Coupled Plasma Mass Spectrometry (ICP-MS)	c.a. 0.08 – 0.27 mg/kg	Sutton et al. 2014
Al, As, B, Ba, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sb, Sr, V and Zn	Pisa, Italy	2014	10 g	36 days	Active	Unwashed and washed samples with tap water and rinsed 3 times with distilled water. All samples were oven dried at 30°C, crushed and homogenized with a mill.	ICP–MS	Ba, Cu, Sb and Zn were trafic-related elements. Mg, Sr and Zn were associated with industrial activities. Fe, Mn, Na and V concentrations were higher in rural/remote areas.	Pellegrini et al., 2014
Ba, Cr, Cu, Fe, Mo, Ni, Pb, Sb, and Zn	São Paulo, Brazil	2015	DNR	Jan 2009 – Feb 2012	Active	Samples were dried at 40 °C and then ground using an agate mill to	For a, Cr, Mo, Sb, and Zn NAA and for Cu, Ni, and Pb ICP- atomic	After of the opening of the highway: Cr and Zn were ten times enriched.	Cardoso-Gustavson et al. 2015

Contaminants	**Location**	**Year**	**Sample mass used (g)**	**Exposition time**	**Type biomonitoring**	**Sample preparation**	**Analysis method**	**Changes or concentrations detected**	**Ref.**
						obtain a fine and homogeneous powder.	emission spectrometry (AES)	Ni, Mo, and Sb showed significant enrichment.	

*RAF: Relative accumulation factor