Air quality biomonitoring of trace elements in the metropolitan area of Huancayo, Peru using transplanted *Tillandsia capillaris* as a biomonitor

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**Abstract:** The air quality and distribution of trace elements in a metropolitan area of the Peruvian Andes were evaluated using transplanted epiphytic *Tillandsia capillaris* as biomonitor. Biomonitor were collected from the non-contaminated area and exposed to five sites with different types of contamination for three months in 2017. After exposure, the content of twenty-one elements were determined by ICP-MS analysis. Datasets were evaluated by one-way ANOVA, exposed-to-baseline (EB), hierarchical cluster analysis (HCA) and principal component analysis (PCA). Results showed significant differences among sampling sites for several elements. According to EF ratios for Ba, Cr, Cu, Pb, Sb, and Zn EB ratios value greater than 1.75 were found around urban areas, indicating anthropogenic influence, which can be attributed to vehicular sources. The highest values of As and Cd were found in areas of agricultural practices, therefore their presence could be related to the employment of agrochemicals (pesticides, herbicides, and phosphate fertilizers). HCA shows that most elements come from vehicular sources and lower from agricultural and natural sources.

**Key words:** active biomonitoring, bioaccumulation, airborne trace elements, Peruvian Andes, *Tillandsia capillaris*.

**INTRODUCTION**

Atmospheric deposition of toxic metals continues being a major concern worldwide because of their negative impact on the environment and human health. In the last decades a significant deterioration of air quality due to urban growing, migration, increasing of vehicles, construction/demolition of roads and buildings, and agricultural practices have been observed in Huancayo city (Haller 2017, Haller & Borsdorf 2013, Milan & Ho 2014). However, due to an unfortunate lack of suitable environmental policies, government disinterest, and economical support, no measurements of air pollution are carried out in this area. Thus, the use of biomonitors widely used in recent years to evaluate the air quality becomes an important contribution to Huancayo city compared to expensive standard techniques (use of semi and automatic High Volume Sampler (Hi-Vol)). Besides, the use of biomonitors allows us the possibility of monitoring many sites simultaneously by short or longer periods of time (Wannaz et al. 2006). Biomonitor can be part of communities of living organisms used to obtain prime information (qualitative and/or quantitative) on aspects of the environment that surround it (Markert et al. 2003). Biomonitoring can be applied through of two methods: active and/or passive biomonitoring. Passive biomonitoring refers to collect organisms
occurring naturally in the ecosystem or within the area of interest and analyze them. In active biomonitoring, the biomonitors can be bred in laboratories or collected of pristine sites for posteriorly be exposed in a standardized form (bag technique) within the area(s) of interest for a defined time period.

Lichens and mosses are two biomonitors widely used and recognized worldwide. However, in recent years several species of the Bromeliaceae family Tillandsia species (“air plants”) have proved also be appropriate biomonitors because they absorb moisture, nutrients, and minerals directly from the atmosphere due to lack root system that avoid the direct contact with the soil (Bermudez et al. 2009, Figueiredo et al. 2007, Wannaz et al. 2013). A large number of these epiphytic Tillandsia species are widely distributed in South America. Regarding the bioaccumulation capacity of atmospheric pollutants by epiphytic Tillandsia genus, Tillandsia capillaris has proved to be an excellent bioaccumulator of trace elements (TE) in the areas surrounding complex polymetallic mining/smelting in Oruro, Bolivia (Goix et al. 2013, Schreck et al. 2016). In Argentina this species has been widely used to determine polycyclic aromatic hydrocarbons (PAHs) (Wannaz et al. 2013), physiological parameters and accumulation of trace elements in different contexts (urban, agricultural, industrial, and mining) (Abril et al. 2014, Bermudez et al. 2009, Mateos et al. 2018, Pignata et al. 2002, Rodriguez et al. 2011, Wannaz et al. 2006). Also, T. capillaris was used to assess the accumulation of PAHs and trace elements in urban, suburban and rural areas in Germany (Rodriguez et al. 2010).

In Peru, studies using biomonitors for the measurement of pollutants in the environment are very scarce. The only study was carried out in Lima using one lichen specie and one specie of Tillandsia genus (Bedregal et al. 2009). Tillandsia capillaris perennial and endemic specie usually found grows on trees, walls, rocks, cables, and electric poles. Hence, the purpose of this study was to investigate the air quality of vicinities urban, peri-urban, and rural areas and trace element distribution using transplanted specimens of Tillandsia capillaris as biomonitors.

MATERIALS AND METHODS

Study area

The present study was undertaken in the Metropolitan area of Huancayo, region Junin (Peru). It is inhabited by 507,075 (INEI 2017) and located up 3200 meters above sea level. Huancayo Metropolitan, a Peruvian mountain city, represents a zone peri-urban in development, nestled in the valleys of the Mantaro River and bordered by mountains that act as natural barriers for air circulation, avoiding the pollutant dispersion. Huancayo city has an important agricultural development in its peripheral areas and it is considered central Peru’s economic and social center. The climate is temperate with an annual mean temperature of 12 °C and precipitation of 517 mm (Huancayo 2017). Western and southwestern winds prevail in this area (Supplementary Material - Figure S1). Four sites were chosen for Tillandsia capillaris transplanting (Figure 1): (a) Huancayo (H, urban area at 3,259 m.a.s.l): The downtown Huancayo, densely populated, where the main source of pollutants is vehicular traffic, (b) Tambo (T, urban area at 3,260 m.a.s.l): neighborhood of the city of Huancayo with similar characteristics of downtown, (c) Chupaca (Ch, agricultural-urban area at 3,263 m.a.s.l): is a peri-urban area (has rural and urban characteristics) situated to 9.7 km southwest of Huancayo. Traffic is medium compared to H and T but has large extensions of agricultural land, and (d) San Agustin de
Cajas (SC, agricultural-urban at 3280 m.a.s.l): located 9.9 km north of Huancayo city, also is a peri-urban area with land used to agricultural practices. Traffic is much less than downtown and is mainly composed of public transport and interprovincial buses.

Transplant experiments

*Tillandsia capillaris* specimens were collected from Eucalyptus tree trunk at Paucara (12° 42’ 0” S, 74° 41’ 0” W), Acobamba Province, Huancavelica (Peru). This area is located to the south of Huancayo city at approximately 138 km of distance and is characterized by having a minimum contact with pollutant emission sources (considered unpolluted site). Net bags containing ~50 g (6-8 plants) were prepared according to Wannaz & Pignata (2006) and transplanted simultaneously (active biomonitoring) to the study area. In each area, seven bags were hung 2.5 m over the ground at different distances. The process of transplantation was held on the 10th September until 22th of December 2017 and corresponds to the spring season with the minor possibility of rains. After the exposure period, plants were collected, stored in paper bags and transported to the laboratory. Baseline element concentrations were obtained analyzing original samples before transplantation. More detail about transplanting sites can be found in Supplementary Material - Table SI.

Major and trace elements determination

First, all samples were dried until constant weight in an oven at 60 ± 2°C and then ground in an agate mortar. About 200 ± 3 mg of each sample in triplicate into a savillex (Teflon bottle) were weighted. Samples were digested using a suprapure mixture of 3.0 mL bi-distilled HNO3 (Duo-PUR, Milestone, USA), 0.5 mL H2O2, and 0.1 mL HF in at hot plate at 250 °C for four hours (Agnan et al. 2013). After digestion achieved, the
samples were cooled, opened and evaporated at 200 °C to beginning dryness. In order to remove remnant HF, 3.0 mL of bi-distilled HNO$_3$ was added and evaporated to beginning dryness three times, and then finally the samples solutions were diluted suitably containing 5% HNO$_3$ into a Falcon tube (15 mL).

Major elements (Al, Ca, Fe, K, and Na) and trace elements (As, Ba, Cd, Ce, Co, Cr, Cu, La, Mn, Ni, Pb, Sb, Sc, Sr, U, V, and Zn) concentrations were analyzed by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS, NexION 300 PerkinElmer, USA). Rh was used as an internal standard to correct data for instrumental drifts and plasma fluctuations. Instrumental conditions for ICP-MS measurements are presented in Table I. All the solutions were prepared with high purity water (18.2 MΩ cm) obtained from a Milli-Q water system (Milli-Q water purification system, Millipore Corp., USA). Analytical curves with six points for each element were used and fitted using linear regression.

Analytical quality control

The accuracy of the analysis was checked using the certified reference material (CRM) of plant SRM 1515 “apple leaves” which was published by National Institute of Standards and Technology (NIST, Gaithersburg, USA). Blank samples were measured in parallel to the decomposition and the analysis of the samples. The recoveries were expressed as the ratio of the concentration measured to certified concentrations of SRM 1515. Major and trace elements concentration were expressed in dry weight (µg g$^{-1}$ DW). All certified elements presented satisfying recoveries in the 86.4-112% range (Table II).

Statistical treatments

Exposed-to-baseline (EB) ratio has been used to evaluate emission sources of the elements measured. The values obtained from this ratio were assessed according to the scale adopted by Frati et al. (2005), where EB ratio values between 0.75 and 1.25 indicate normal conditions in the environment; while 1.25 < EB < 1.75 indicate accumulation, and EB ratio > 1.75 indicates severe accumulation of pollutants, which may be related to anthropogenic emission sources.

In order to identify possible groups of elements as tracers of natural or anthropogenic sources of the elements measured in the T. capillaris samples, hierarchical cluster analysis (HCA) with Ward’s method was used. All statistical analysis in this study was performed by the CRAN R (R Team Core 2019) free language through of the following packages ggplot2 (Wickham & Chang 2016), dplyr (Wickham et al. 2017), and ClusterofVar (Chavent et al. 2012).

Table I. Instrumental conditions for ICP-MS measurements.

| ICP-MS conditions       | Values |
|-------------------------|--------|
| RF power                | 1150 W |
| Frequency               | 27.2 MHz |
| Plasma gas flow rate    | 11.5 L min$^{-1}$ |
| Auxiliary gas flow rate | 0.55 L min$^{-1}$ |
| Nebulizer gas flow rate | 0.97 L min$^{-1}$ |
| Sample uptake rate      | 0.6 mL min$^{-1}$ |
| Measurement mode        | Dual (PC/analog) |
| Acquisition time        | 1 s    |
| Dwell time              | 200 ms |
| Replicates              | 6      |
RESULTS

Trace and major elements concentration in T. Capillaris

The arithmetic means, standard deviations, ranges of concentrations and ANOVA results of the elements measured in T. capillaris transplanted at the four sites and baseline site are shown in Table III. Overall element concentrations decreased in the following order: Al > Ca > K > Fe > Na > Mn > Ba > Zn > Sr > Cu > Pb > As > Ce > Cr > La > Ni > Co > V > Sb = Sc > Cd. In general, in most transplanted sites the biomonitors showed the highest concentration values of the elements measured than baseline samples, confirming the pollutants accumulation capacity of this bromeliad specie. A statistically significant difference (p < 0.05) among exposure sites for Ba, Ca, Cd, Cu, Fe, Pb, Sb, and Zn were observed, while significant differences (p < 0.01) were found for Al, As, Cr, and Na. No significant differences were observed for K, La, Mn, Ni, Sc, Sr, and V. Highest concentration values of Ba, Cr, Cu, Fe, Pb, Sb, V, and Zn were observed in the urban areas (H and T) than peri-urban areas (Ch and SC). By contrast, the highest values of As and Cd contents were found in Ch and SC sites. These areas have peri-urban characteristics, where residential areas and agricultural areas coexist. Moreover, higher Ca content was found in H, and Ch sites. Like calcium in considered a biomarker of cement production (Abril et al. 2014, Ferreira et al. 2012), the abundant Ca found is probably released from local construction activities in form of waste and crustal dust resuspension.
EB ratios

The EB ratios calculated as described in section statistical treatments are shown in Table IV. According to the scale of Frati et al. (2005), values greater than 1.75 are indicative of anthropogenic influence. In this case, As, Ba, Ca, Cd, Cr, Cu, Pb, Sb, V, and Zn showed EB ratios > 1.75 for most exposure sites. While the whole study area presents six elements (As, Cd, Cu, Pb, Sb, and Zn) with EB ratios > 2.00, suggesting a strong influence of these elements in the environment of Huancayo city.

Distribution maps of the elements with EB ratios > 1.75

The distribution map of As, Ca, Cd, Pb, Sb, and Zn are shown in Figures 2 to 7. In these maps, darker areas are indicative of the presence of the element in higher concentrations.
Hierarchical cluster analysis

The dataset of the elements submitted to hierarchical cluster analysis yielded two distinct groups (Figure 8). K, Sr, Na, Mn, Ni, La, Sc, Al, Ce, V, and Co are grouped in Group 1, while Group 2 includes Zn, Cu, Fe, Pb, Ba, Sb, Cr, Ca, Cd and As (closely associated among themselves). The dendrogram indicates that elements belonging to the same group may have the same origin. As it is seen, the Group 1 contains elements related to natural sources; while in Group 2 can be observed elements of anthropogenic origin.

DISCUSSION

The EB ratio values obtained for As (T, Ch, and SC sites), Ba (H and T), Ca (H, T, and Ch), Cd (all sites), Cr (H, T, and Ch), Cu (all sites), Pb (all sites), Sb (all sites), V (H and T), and Zn (all sites) were all greater than 1.75, suggesting severe influence of anthropogenic sources in the Metropolitan area of Huancayo. Considering the whole study area was observed accumulation (1.25 < EB ratios < 1.75) of Ca, Ce, Fe, La, Ni, and V. These results suggesting the influence of soil particles in minor proportion than anthropogenic origin. For example, Ca enrichment is probably related
to both, local construction dust activities and crustal dust resuspension. Likewise, Ce and La, two lanthanides used in the manufacturing of catalytic converters (Rached et al. 2018) for the control of vehicle emission are also released into the environment and there is evidence that their concentration in soils and plants exhibit cytotoxic effects, causing a decrease in root elongation (Kotelnikova et al. 2019).

Significantly EB ratios > 1.75 were found for As, Cd, Cu, Pb, Sb, and Zn confirming their anthropogenic origin of these elements, while Al, Co, K, Mn, Na, Sc, and Sr in the whole study area was found to normal conditions (EB ratios < 1.25) in the environment, which could indicate natural origin (Table IV).

In the literature, Cd, Cu, Pb, Sb, and Zn are considered toxic elements (Cooper and Harrison 2009, Hoodaji et al. 2012, Yaman 2006) being that these elements usually are released from vehicles through vehicular exhaust emissions, and both tire and brake wear in urban areas (Pellegrini et al. 2014, Rodriguez et al. 2011). Effectively, these elements presented higher concentration values around urban areas (T and H sites) from Huancayo city (Table III), where car congestion happens every day and during all day.

On the basis of EB ratios, Pb can cause severe effects on human health, especially among children (USEPA 1999). Peruvian vehicles no longer use leaded gasoline after it was banned in 2009 (Onursal 1997). However, Pb still presents in the environment of the Metropolitan area of Huancayo can be due to past emissions (contaminated soils, water pipe debris) and the fast increase of Huancayo’s vehicle population. For instance, Pb is widely used in a number of car components such as lead-based paint, lead wheel weights, lead-acid batteries and solder in electronics (Song et al. 2012). Similar conclusions were reported by Vianna et al. (2011) who assessed the Pb concentration in two Metropolitan areas (Rio de Janeiro and Salvador) from Brazil. Arsenic is ubiquitous in the environment and until the 1970s approximately 80% was used to the manufacturing of arsenical pesticides (mostly as sodium arsenite). Actually, due to its toxicity, the use of As for manufacturing of pesticides decreased by about 50%, however organic As compounds still dominate the production of pesticides (Kabata-Pendias, 2010). Cadmium emissions arise from either natural or anthropogenic sources, including volcanic emissions, smelting, and refining of nonferrous metals, fossil fuel combustion, iron and steel industry, municipal waste incineration and the use of fertilizers derived from rock phosphate (WHO 2000). Like higher EB ratios values from As and Cd were found in Ch and SC sites, where huge tracts of land are used for the production of several crops, we may assume that elevated concentrations of As and Cd are related to the intense use of pesticides in agricultural activities.

The distribution map of As (Figure 2) shows high levels of this element to the southwestern and relatively elevated to the north. In these places are located the sites Ch and SC, where the land is used to agricultural practices for production of different types of crops and vegetables such as vegetables, corn, potatoes, onions among others.

The results of Ca concentrations in T. capillaris are illustrated in Figure 3. Calcium element is considered a biomarker for cement production (Abril et al. 2014, Ferreira et al. 2012). As it is seen, calcium element is widely distributed in almost all study areas with the higher content of this element being observed in Ch, T, and H sites. In recent years, both T and H sites suffer a transformation with the demolition of roads and the historic “Plaza Constitution” located in downtown and the construction of three new
supermarkets, while Ch site is facing the built of residential buildings and bridges (Haller 2014, Haller & Borsdorf 2013). Despite, the contact with this element may cause cancer (Koh et al. 2011, Krejcirikova et al. 2018), dust pollution (Zuo et al. 2017) and environmental impact (Chen et al. 2015), construction activities on this area are poorly controlled.

The highest contents of Cd were detected in the north and southwestern zone from downtown (Figure 4), specifically in the two peri-urban areas (Ch and SC) where agricultural activities are developed by farmers. Cadmium occurs naturally in the environment usually linked to zinc (Lambert et al. 2007). Cadmium usually is emitted by smelting refining of nonferrous metals, fossil fuel combustion, metalliferous mining, incineration waste, phosphate fertilizer production, and by industries using cadmium in rechargeable batteries, pigments, electroplating, solar cells, and as plastic stabilizers. Cadmium in agricultural soils can enter via phosphate fertilizers (0,1-170 mg/kg) (Kabata-Pendas 2010, Kratz et al. 2016). As the land use in areas around from Metropolitan area of Huancayo is considered suitable for the production annual and biennial of crops (Haller & Borsdorf 2013), a big quantity of Cd may be related to the use of agrochemicals (pesticides, fertilizers, and herbicides).

Lead is a toxic element even at low concentration and is related to vehicular emissions and dust re-suspension (Wani et al. 2015). In Peru, leaded gasoline was banned in 2009 (Bekir Onursal, 1997), however, the distribution map of Pb (Figure 6) shows that Pb contamination is still present in this area. A similar result has been reported by De la Cruz et al. (2009) who studied the lead concentrations and its isotope ratios in samples of particulate matter ($PM_{10}$) before and after non-leaded fuel normative in Zaragoza (Spain) and concluded no-statistically significant decrease in the average concentration of lead. The higher Pb levels were found in Ch, H and T (the place where exist a gas station approximately twenty years ago).
No information about this element was found in the literature for comparison purposes or to affirm the decreasing of this element compared to previous years.

The distribution map of Sb (Figure 8) shows three sites: two urban areas (T and H) and a rural/urban area (Ch) with higher Sb content. Antimony is a metalloid and occurs naturally as trace elements in the environment (soils) (Sanchez-Rodas et al. 2017, Wilson et al. 2010), however, in the last decades this element was associated with traffic due to that several parts of vehicle contain Sb alloys and other Sb compounds (Fujiwara et al. 2011, Hu et al. 2015, Sanchez-Rodas et al. 2017). According to EB ratios and the location, we can conclude that Sb comes from vehicular sources.

The distribution map of Zn (Figure 7) revealed elevated content of this element in the southeastern, around urban areas, which indicates anthropogenic origin. The highest levels were observed in downtown (H) and its neighbor Tambo city. Both areas have a big circulation of vehicles all day causing heavy traffic.

The hierarchical cluster analysis (Figure 8) helps us to elucidate our analysis. The dendrogram shows two groups: the first-formed mainly by elements released from natural origin (K, Sr, Mn, Na, Ni, La, Sc, Al, Ce, V, and Co), while the second group was represented by As, Cd, Ca, Cr, Sb, Ba, Pb, Fe, Cu, and Zn elements probably released from anthropogenic activities.

Potassium, Na, Sr, Al, Sc, and Mn in the group show EB ratios > 1.25 (Table IV) and, therefore, geogenic sources seem to prevail for this element association. Manganese (Mn) is one of the most abundant trace elements derive from the lithosphere. Besides, Mn oxides are considered to be the most abundant compounds of the Earth’s crust (Kabata-Pendias 2010). Manganese usually is used to
provide hardness and toughness for steel and various alloys manufacturing, for production of pigments, ceramics, and glass. Likewise, Manta et al. (2002) supported a natural origin of Mn, Ni, Co, and V in urban soils of Italy, and Ventura et al. (2017) reported that Na, K, and Al elements were released from natural sources in urban areas from Rio de Janeiro, Brazil.

As it is observed in Figure 8, As and Cd are closely related, suggesting that both come of similar sources. In this work, hypothesized that the presence of these elements could be related to a wide array of agricultural applications as fertilizers and fungicides. Fe is emitted by industrial and urban pollution sources (Speak et al. 2012). For instance, Fe-rich particles are commonly released by traffic car and buses through brake-disc wear, brake-pad, and corrosion of car-body parts (Miranda et al. 2016, Penkala et al. 2018).

CONCLUSIONS

The *Tillandsia capillaris* behaved as an effective biomonitor for assessment of air quality in the Metropolitan area of Huancayo. The results of the study show the impact of anthropogenic sources in the study area. Concerning the association between the trace elements and the different anthropogenic activities, highest levels of Cu, Pb, Sb, Zn were found to be related to vehicular emissions, while As and Cd content were found higher in peri-urban areas, relating these elements to the employment of agrochemicals, while high content of Calcium was observed near construction/demolition areas.
Figure 5. Distribution map of Pb concentrations.

Figure 6. Distribution map of Sb concentrations.
Figure 7. Distribution map of Zn concentrations.

Figure 8. Results of the hierarchical cluster analysis (dendrogram) of the elements measured in T.capillaris.
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SUPPLEMENTARY MATERIAL

Figure S1. Wind roses from September to December 2017, obtained from geophysical institute of Peru, Chupaca (12° 2’ 18” S; 75° 20’ 17” W).

Table S1. Characteristics of each transplanting point carried out in the Metropolitan area of Huancayo.

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An Acad Bras Cienc (2020) 92(1) e20180813 16 | 17
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ARHD works on paper design, data interpretation, writing, and review. AFO worked on the writing essay and methodology. RWHD worked on methodology and data interpretation. JLL worked on data interpretation. AG contributed to the critical review of the results and text and for support to the chemical analysis.