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Research Article

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Biomonitoring of atmospheric trace elements in agricultural areas and a former uranium mine

Abstract: Most biomonitoring studies worldwide have evaluated the air quality in industrial and urban areas, and even in mining areas to a lesser extent. However, air quality investigations in agricultural areas are scarce. In the present study, the trace metal accumulation and physiological response of the biomonitor *Tillandsia capillaris* were assessed. Plant samples were transplanted to a reference site, a former open-cast uranium mine, and agricultural sites with varying pollution levels (from normal agricultural practices and near an open rubbish dump) in the province of Córdoba, Argentina. Biomonitors were exposed to ambient air for different exposure periods for physiological or trace element determination. The bioindicators revealed that the highest physiological damage occurred at the sites close to the open dump and the former uranium mine, while a comparison among exposure periods indicating the winter season produced the highest physiological damage in the biomonitor due to the adverse climatic conditions and air pollution. As the trace metal accumulation in the biomonitor was mainly associated with the open dump and uranium mine sites, monitoring and remediation programs should now be applied to these sites in order to alleviate the negative effects of pollution on the environment and the population.

Keywords: air quality; *Tillandsia capillaris*; open rubbish dump; trace metals; mine

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1 Introduction

Historically, urban and industrial growth has been accompanied by a significant degradation of the environment, caused by pollutant emissions in all regions of the world. Although major air pollution problems have been reported in urban and industrial centers, pollution can also be found at other sites due to agricultural activities and the atmospheric long-distance transport of pollutants [1]. Air pollution studies have mainly focused on urban and industrialized areas [2-5] and also on regions impacted by road transport [6], while investigations are scarce in rural areas [7].

In recent years, the continuous rise in emissions has caused growing concern over pollution by persistent toxic pollutants, such as heavy metals, which are deposited in soils, water, crops, and natural vegetation [8-11]. Although trace metals are natural components of rocks and soils, many anthropic activities such as mining, smelters, and other industrial and agricultural processes release trace metals to the environment. Various studies have reported the negative impact of heavy metals in agricultural areas, in which pollutants have accumulated in edible crops, implying a toxicological risk for humans and livestock through food and seed consumption [10; 12-15].

The province of Córdoba has an extensive agricultural region of 4,850.000 ha; the main crops are soybean, corn, wheat, sunflower and sorghum (from the government of Córdoba database http://web2.cba.gov.ar/produccion/sayg/paginas/pag-secundarias/caracterizacion/prod-agr.htm). Recently, studies performed on soybean, wheat and sorghum have shown that these crops can accumulate higher levels of toxic metals than others, thus putting the food chain at risk [10,15-21]. The evaluation of mining areas is also necessary, as some studies have reported harmful environmental effects occurring from mines that are no longer active [22-23].

Taking into account these facts, it is necessary to assess the atmospheric quality of agricultural and mining areas in order to evaluate possible anthropic sources affecting air and crop quality. Biomonitoring is an effective and economic technique, applicable both locally and regionally, and is recommended by European legislation [24-27]. Many biomonitoring studies have been carried out using epiphytic species of the *Tillandsia* genus; these have shown a great capacity for metal accumulation, mainly due to their morphological and functional properties. For example, the trichomes can absorb nutrients directly from the atmosphere by wet or dry deposition [28-33]. Although most biomonitoring studies performed with *Tillandsia* species have evaluated the atmospheric quality through the accumulation of metals, some studies have investigated the physiological status when these indicators were exposed to different pollution levels [25; 34-35]. The physiological parameters that have been used to assess pollution damage in bioindicators include variation in the content and degradation of chlorophyll [36-37], increases in membrane leakage and peroxidation of membrane lipids [38-39] and accumulation of various air pollutants such as heavy metals [5; 34; 40].

In Córdoba, passive and active biomonitoring studies using *Tillandsia* species have been able to identify strong physiological damage in the bioindicator in the vicinity of agricultural areas [36; 40-42].

Therefore, the purpose of this study was to evaluate the air quality in agricultural and mining areas through a biomonitoring study employing *Tillandsia capillaris* as the active biomonitor.

2 Methods

2.1 Biological material, collection procedures and sample preparation

Plants of *Tillandsia capillaris* Ruíz & Pav. form *capillaris* were collected from tree trunks of similar exposures in San Isidro, Córdoba Province, Argentina (31º 48’ 55’’ S, 64º 24’ 04, 7’’ W), which represented the baseline material at the collection site (used as reference). This area is characterized by low emissions of air pollutants.

Polyethylene mesh bags (mesh size of 1x1 cm) containing 300 g of *T. capillaris* (approximately 10 individuals) were prepared according to Bermudez et al. [36] and simultaneously transplanted to three agricultural areas with different potential anthropic emission sources of pollutants, to another area with a former open-pit uranium mine, and to a reference site. The bags were placed in a rotating system, which allowed adequate exposure of the samples with respect to the wind direction. In each area, one or more transplanting systems containing four bags each were placed at 3 m above ground level [34].

Four periods of three month exposures (August-October 2005; November 2005-January 2006; February-April 2006; May-July 2006) and six month exposures (August-January and February-July during 2006 and 2007) were performed for physiological analyses and trace metal accumulation, respectively. The difference in exposure time was chosen based on previous studies, to evaluate the seasonality and accumulation of pollutants in the epiphyte [35].

After exposure, one bag was retrieved from each study area, and part of the sample was separated to determine the water content. Another fraction was stored in plastic vials at -15 °C in complete darkness and used for physiological determinations. The remaining material was prepared for metal determinations.

In order to establish the initial state of the samples before transplantation physiological variables were measured in the baseline material and then compared with those corresponding to the transplanted material at the reference site, with the objective of evaluating a potential transplant effect. The results of the analysis of variance showed significant differences (data not shown) so samples transplanted to the reference site were chosen for subsequent analysis.

2.2 Exposure areas

The study region included five areas in Córdoba province, central Argentina (Fig. 1; Table 1). Here, the land morphology is highly variable, ranging from a mean altitude of approximately 450 m.a.s.l. in the southeast to more than 1,600 m.a.s.l. in the west. The climate is mild, with an annual mean temperature of 15 °C and an average annual rainfall of 500-900 mm, depending on the elevation. Figure 2 shows the climatic conditions of the exposure periods for the physiological analyses and trace element accumulation.

The exposure areas selected and their characteristics were as follows:

- Agricultural area I: an agricultural site located in Despeñaderos town, 45 km south of Córdoba city and with a population of 7,000 inhabitants. Soybean, maize and wheat are the main crops grown at this site.
- Agricultural area II: an agricultural area located in the towns of Pozo del Tigre and Estación General Paz, 32 km north of the city of Córdoba. In these areas, the main crops are soybean, sorghum and maize.

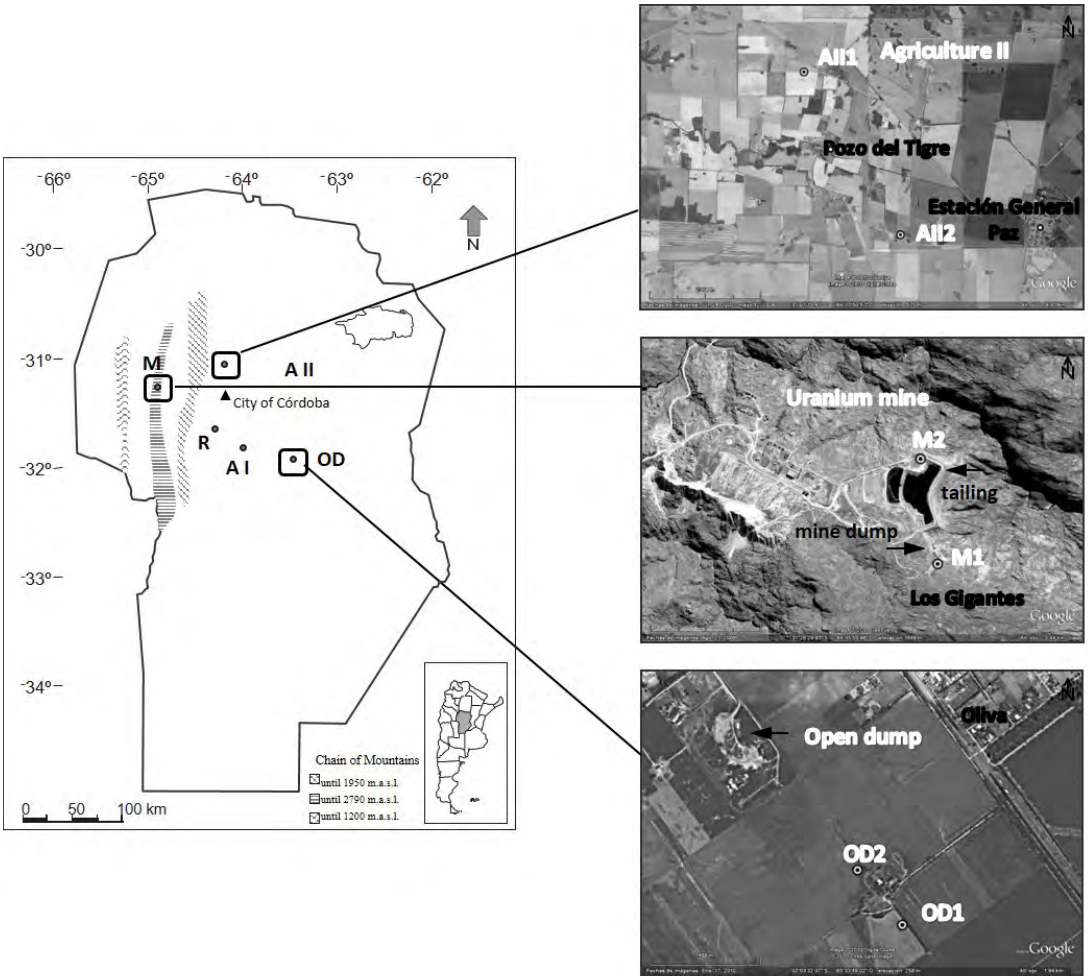

Figure 1: Biomonitoring exposure areas in Córdoba, Argentina.

Table 1: Environmental characterization of exposure sites.

Exposure areas	Exposure sites	Distance to major emission sources	Location Latitude / Longitude
Reference	**R 1**	-	31º 48´55´´ S/ 64º 24´04.7´´ W
Former open-cast uranium mine	**M 1**	Tailing dam 0.2 km Remediation area 0.4 km Mine dump 0 km	31º 26´41.1´´ S/ 64º 45´41.9´´ W
	M 2	Tailing dam 0.04 km Remediation area 0.1 km Mine dump 0.5 km	31º 26´25´´ S/ 64º 45´44.8´´ W
Agricultural area I	**AI 1**	-	31º 49´03.3´´ S/ 64º 16´12.4´´ W
Agricultural area II	**AII 1**	-	31º 06´15.3´´ S/ 64º 11´37.6´´ W
	AII 2	-	31º 08´09.7´´ S/ 64º 10´19.2´´ W
Open dump	**OD 1**	Open- rubbish dump 1 km	32º 03´46.5´´ S/ 63º 33´42´´ W
	OD 2	Open- rubbish dump 0.7 km	32º 03´38.7´´ S/ 63º 33´49.6´´ W

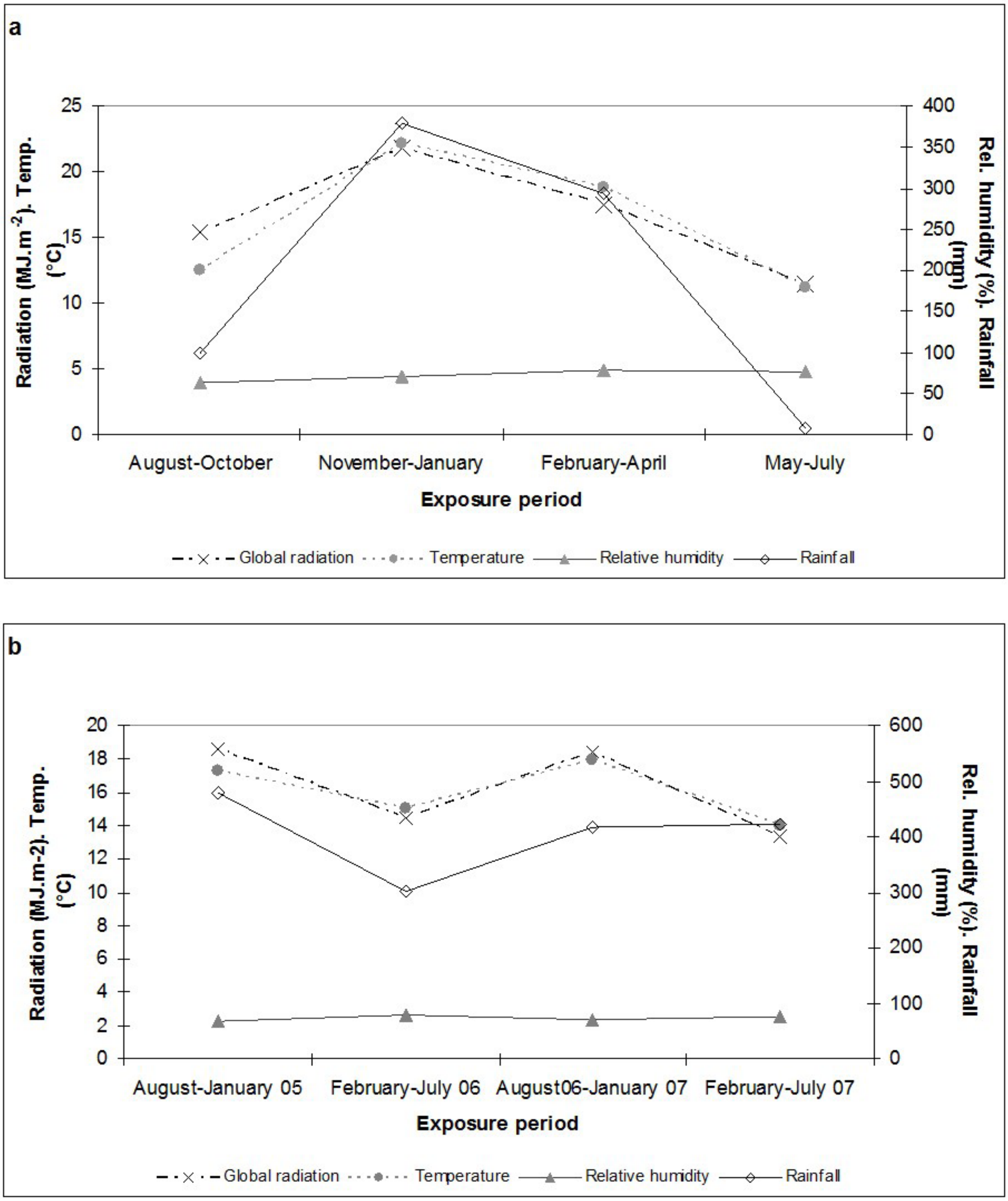

Figure 2: Climatic conditions of the exposure periods for physiological and trace metal determinations.

- Open rubbish dump area: an agricultural area with a population of 13,000, located in the town of Oliva, 96 km southeast of Córdoba city in the Chaco-Pampean plain. This exposure area is characterized by the presence of soybean, wheat, sorghum, maize and livestock. The main feature of this area is the location of a 16-hectare open rubbish dump, where municipal wastes are burned and buried. In Argentina, over 90% of municipalities do not have an adequate waste management, and waste dumps are a normal practice [43].
- Open-cast uranium mine: this former uranium mine is located in the Sierras Grandes mountains, 100 km west of Córdoba city (1,600 m.a.s.l.). Currently, about 1.6 million tons of mining waste and marginal grade mineral - as well as 2.4 million tons of heap leach residues - are deposited there [44]. The mine is under the supervision of the Uranium Mining Environmental

Restoration Project (PRAMU) of the National Atomic Energy Commission of Argentina (http://www.cnea.gov.ar/politica_ambiental/pramu.php). It is important to note that previous studies have detected high levels of uranium in both biomonitors [42] and topsoils [44].

– Reference area: this corresponded to the collection site of *T. capillaris*, which was located at San Isidro, 80 km south of Córdoba city. This area is part of the Espinal Phytogeographic region, where *Prosopis* spp., *Geoffroea decorticans,* and the shrubs *Larrea* spp., *Acacia caven* and *Condalia microphylla* are common. There are no crops near the exposure area, which is characterized by low emissions of air pollutants and represents the initial (baseline) condition of this species.

2.3 Sampling procedure and chemical analysis

After each exposure period, three sub-samples of each sampling bag (*n* = 12: 4 bags x 3 sub-samples) were taken at each of the exposure sites corresponding to the exposure areas (Table 1). Determination of chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), malondialdehyde (MDA), hydroperoxy-conjugated dienes (HPCD), sulfur content (S), dry weight/fresh weight ratio (DW/FW) and foliar damage index (FDI) was performed as previously described by Pignata et al. [42]. The concentrations were expressed as dry weight (g^{-1} DW). In addition, the relative electrical conductivity (EC r) was calculated according to Tarhanen et al. [45] in five sub-samples, and expressed as fresh weight (g^{-1} FW).

The concentrations of K, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Br, and Pb were determined in leaves of *T. capillaris* (3 g DW) according to Rodriguez et al. [34]. Briefly, plant material was ground and reduced to ashes at 450 °C for 4 hours, followed by digestion with HCl (18%): HNO_3 (3:1). The solid residue was separated by centrifugation and the volume adjusted to 25 mL with Milli-Q water. Then, 10 ppm of a Ge solution was added as an internal standard. Aliquots of 5 µL were taken from this solution and dried on an acrylic support. The samples were measured for 200 s, using the total reflection set up mounted at the X-ray fluorescence beamline of the National Synchrotron Light Laboratory (LNLS), Campinas, Brazil. As a quality control, blanks and samples of the standard reference material “Hay IAEA-V-10” were prepared in the same way and run after five determinations to calibrate the instrument; the results were found to be within ± 5% of the certified value. The coefficient of variation of the replicate analysis was calculated for different determinations, and the variations were found to be less than 10%. Standard solutions, with known concentrations of different elements and Ge as the internal standard, were prepared for calibrating the system.

Quality control results obtained from the analysis of standard reference material are shown in Table 2.

2.4 Statistical analysis

Results were expressed as the mean value ± standard deviation of three determinations for each of the bags at the exposure sites of each exposure area. The data of the physiological parameters and trace element concentrations were submitted to a one-way analysis of variance (ANOVA). A pairwise comparison of means by the Least Significant Difference test was carried out whenever the ANOVA indicated significant effects ($p<0.05$), with the ANOVA assumptions being previously verified graphically (residual vs. fitted values, box plots, and stem leaf plots) and numerically (Shapiro–Wilks test for normality and the Levene test for equality of variances).

A Principal Component Analysis (PCA) was performed in order to examine the results in relation to the trace element accumulation in the biomonitor, by transforming the original set of variables into a smaller set of linear combinations that takes into account most of the variance of the original data set. A PCA analysis was made using the sampling sites as the classification criteria in order to

Table 2: Quality control results (µg g^{-1} DW) obtained from the analysis of Hay IAEA-V-10 and Lichen IAEA-336 by X-ray fluorescence.

Element	Reference material	
	Certified	Experimental
Br	8 ± 4	7.7 ± 2.8
Ca	21600 ± 1200	21505 ± 900
Co	0.13 ± 0.03	0.12 ± 0.04
Cu	9.4 ± 0.9	8.9 ± 0.78
Fe	186 ± 13	151 ± 33
K	21000 ± 2900	19500 ± 2700
Mn	47 ± 7	39.9 ± 3.5
Ni	4.2 ± 1.1	3.68 ± 0.49
Pb	1.6 ± 1.1	1.75 ± 0.40
V*	1.47 ± 0.44	1.18 ± 0.65
Zn	24 ± 2	23.1 ± 1.3

Mean of five replicates. Vanadium (V) values were calculated using IAEA-336 Lichen samples.

assess the relationship with the physiological biomonitor response. It should be noted that the assumptions of PCA were met (with continuity of the variables and that the number of elements observed being greater than the number of original variables). In addition, element concentrations for exposed to basal ratios (EB) were calculated according to Wannaz and Pignata [35] and Frati el al. [46], and the statistical analysis was performed using the InfoStat software (student version 2012; National University of Córdoba, http://www.infostat.com.ar/index.php).

3 Results

3.1 Physiological parameters

Table 3 shows the mean values of the physiological parameters measured in *T. capillaris* and the ANOVA results. In general, the highest concentrations of total chlorophyll in the biomonitors were found in the open dump area. The comparisons among different exposure periods revealed lower chlorophyll concentrations in the epiphytes exposed in the period between May - July, corresponding to the cold and dry season (Figure 2a).

In regards to the membrane lipid oxidation products (HPCD and MDA), the comparison among exposure sites in general showed the highest values at the open dump and mining sites, while the comparison among periods did not reveal a clear response pattern. Furthermore, the values of sulfur content were in general significantly higher in the open dump area and during the period between November and January.

The Chl-b/Chl-a ratio revealed a degradation of chlorophyll-a due to air pollution [47-49], but did not show a clear response pattern among exposure sites. Higher values of chlorophyll degradation were found at the open dump site in the period between August and October, and at the reference site in the exposure period of February-April. It is noteworthy that in the reference site, chlorophyll-a degradation could be due to specific differences in climatic conditions compared to the other sites, because this site has no sources of contamination.

The highest values of DW/FW ratios were detected at the former uranium mine, with the comparison among exposure periods showing the greatest values in the period of November-January.

In contrast, the relative EC only revealed significant differences among exposure sites between May and July, the period in which the highest values were also found at agricultural site I. However, the comparison among exposure periods showed the lowest values between May and July, which coincided with the minimum temperatures of this study. Finally, the foliar damage index (FDI), which combines the changes in each individual stress parameter [41], revealed the highest values of FDI at the open dump and mining sites. Comparison among exposure periods generally indicated the winter period was the one with the highest FDI values.

3.2 Accumulation of trace elements in the biomonitor

Results of PCA analysis are presented in biplots (Fig. 3 a-b), with the eigenvalues corresponding to the first three components shown in Table 4. This analysis was carried out using exposure sites as classification criteria, in order to assess their relationships with the trace element contents in the biomonitor. The contents of V, Mn, Fe, Co, Zn, Br and Pb were associated with the open dump and agriculture-I sites, while contributing to the first component. The second component was made up of contributions from the Ni and Cu contents, which were also associated with the former uranium mine. The trace elements K and Ca contributed to the third component, and were associated with the reference and agriculture-II sites respectively.

In addition, an exposed to basal ratio (EB) was calculated in samples of *T. capillaris*, which was performed with the purpose of evaluating pollutant emission sources (natural or anthropogenic). The EB ratio for each element was calculated according to the following equation:

$$EB = Xi / Xb$$

where Xi is the concentration of element X in mg g^{-1} DW in the sampling area i, and Xb is the concentration of element X in mg g^{-1} DW in the baseline samples collected at the reference area. Taking into account that elements with EB values near unity may indicate a natural origin, we considered only values greater than 2 to reflect a potential anthropogenic source in this study. The mean values of EBs are presented in Fig. 4, which showed vanadium enrichment in samples corresponding to the open dump and agriculture-II sites of 2.30 and 2.34 respectively. Moreover, nickel enrichment (2.15) was observed in the former uranium mine.

4 Discussion

4.1 Physiological response in *T. capillaris*

The increase of chlorophylls in the biomonitors corresponding to the open dump area may be explained as a result of a moderate fertilization by pollutants such

Table 3: Mean values (± standard deviation, SD) and results of the analysis of variance (ANOVA) of the total chlorophylls (Chl-a+b), hydroperoxy conjugated dienes (HPCD), malondialdehyde (MDA), sulfur (S), chlorophyll-b/chlorophyll-a ratio (Chl-b/Chl-a), dry weight/fresh weight ratio (DW/FW), relative electrical conductivity (EC r) and foliar damage index (FDI) measured in *Tillandsia capillaris* at different exposure periods and sites.

	Mean ± SD					ANOVA[a]
	Reference	Uranium mine	Agriculture I	Agriculture II	Open dump	
Chl-a+b (mg.g^{-1})						
Aug-Oct	1.666 ± 0.183 b A	1.788 ± 0.139 b A	1.234 ± 0.263 c B	1.666 ± 0.214 b A	2.384 ± 0.302 a A	***
Nov-Jan	1.371 ± 0.101 b B	0.925 ± 0.084 c B	1.407 ± 0.094 b A	1.603 ± 0.186 a A	1.617 ± 0.077 a B	***
Feb-Apr	1.680 ± 0.110 b A	0.582 ± 0.029 e C	0.925 ± 0.085 d C	1.161 ± 0.251 c B	2.434 ± 0.186 a A	***
May-Jul	1.318 ± 0.126 a B	0.364 ± 0.101 c D	0.322 ± 0.083 c D	0.302 ± 0.202 c C	0.571 ± 0.114 b C	***
ANOVA[b]	***	***	***	***	***	
HPCD (μmol.g^{-1})						
Aug-Oct	23.78 ± 5.585 d B	72.12 ± 7.830 a A	32.31 ± 4.943 c A	40.23 ± 10.49 b A	64.68 ± 6.967 a A	***
Nov-Jan	31.80 ± 4.361 b A	24.19 ± 3.820 c C	36.03 ± 5.210 a A	32.87 ± 5.881 b B	34.94 ± 4.974 b C	***
Feb-Apr	30.05 ± 3.150 b A	21.09 ± 2.401 c C	24.68 ± 3.452 c B	30.53 ± 3.992 b B	33.30 ± 4.170 a C	***
May-Jul	24.54 ± 6.449 c B	36.39 ± 5.416 ab B	31.56 ± 5.718 b A	33.76 ± 4.824 b B	42.41 ± 6.357 a B	***
ANOVA[b]	***	***	***	***	***	
MDA (μmol.g^{-1})						
Aug-Oct	0.140 ± 0.014 cd B	0.329 ± 0.035 a A	0.161 ± 0.013 c AB	0.129 ± 0.035 d B	0.185 ± 0.023 b B	***
Nov-Jan	0.167 ± 0.008 b A	0.355 ± 0.047 a A	0.144 ± 0.011 b BC	0.140 ± 0.032 b B	0.157 ± 0.016 b BC	***
Feb-Apr	0.102 ± 0.017 C	0.128 ± 0.005 B	0.138 ± 0.015 C	0.139 ± 0.023 B	0.133 ± 0.008 C	ns
May-Jul	0.142 ± 0.013 c B	0.137 ± 0.014 c B	0.175 ± 0.037 c A	0.345 ± 0.052 b A	0.545 ± 0.062 a A	***
ANOVA[b]	***	***	**	***	***	
S (mg.g^{-1})						
Aug-Oct	1.186 ± 0.202 d B	1.531 ± 0.322 cd B	1.734 ± 0.045 c B	2.378 ± 0.470 b A	2.907 ± 0.466 a A	***
Nov-Jan	2.004 ± 0.100 c A	2.050 ± 0.068 c A	2.099 ± 0.167 bc A	2.329 ± 0.262 a A	2.256 ± 0.216 ab B	***
Feb-Apr	1.285 ± 0.110 bc B	1.152 ± 0.069 c C	1.208 ± 0.100 c C	1.390 ± 0.222 ab B	1.479 ± 0.049 a C	***
May-Jul	0.427 ± 0.147 d C	0.561 ± 0.126 cd D	0.673 ± 0.126 bc D	0.794 ± 0.122 b C	0.997 ± 0.239 a D	***
ANOVA[b]	***	***	***	***	***	
Chl-b/Chl-a						
Aug-Oct	0.638 ± 0.037 bc B	0.671 ± 0.028 ab AB	0.625 ± 0.019 c C	0.625 ± 0.020 c	0.695 ± 0.063 a A	***
Nov-Jan	0.623 ± 0.017 B	0.648 ± 0.032 B	0.628 ± 0.015 BC	0.655 ± 0.053	0.653 ± 0.043 AB	ns
Feb-Apr	0.697 ± 0.058 a B	0.635 ± 0.036 cd B	0.688 ± 0.038 ab A	0.669 ± 0.043 bc	0.592 ± 0.105 d B	***
May-Jul	0.737 ± 0.063 A	0.695 ± 0.052 A	0.677 ± 0.095 AB	0.643 ± 0.076	0.628 ± 0.047 AB	ns
ANOVA[b]	***	*	*	ns	*	
DW/FW						
Aug-Oct	0.319 ± 0.076 bc C	0.379 ± 0.065 ab C	0.401 ± 0.055 a AB	0.334 ± 0.029 b B	0.216 ± 0.017 c C	***
Nov-Jan	0.485 ± 0.055 b A	0.690 ± 0.076 a A	0.430 ± 0.067 c A	0.370 ± 0.047 d A	0.322 ± 0.025 d A	***
Feb-Apr	0.266 ± 0.034 d D	0.535 ± 0.020 a B	0.415 ± 0.011 b A	0.352 ± 0.019 c AB	0.216 ± 0.018 e C	***
May-Jul	0.381 ± 0.014 a B	0.355 ± 0.051 ab C	0.356 ± 0.013 ab B	0.344 ± 0.019 b B	0.296 ± 0.009 c B	***
ANOVA[b]	***	***	*	*	***	
EC r						
Aug-Oct	0.260 ± 0.131 A	0.172 ± 0.120 AB	0.211 ± 0.076	0.245 ± 0.190 A	0.107 ± 0.039	ns
Nov-Jan	0.226 ± 0.050 A	0.187 ± 0.061 AB	0.128 ± 0.050	0.218 ± 0.068 AB	0.207 ± 0.053	ns
Feb-Apr	0.258 ± 0.057 A	0.247 ± 0.099 A	0.240 ± 0.151	0.281 ± 0.053 A	0.195 ± 0.181	ns
May-Jul	0.103 ± 0.052 bc B	0.047 ± 0.008 d B	0.177 ± 0.031 a	0.126 ± 0.032 b B	0.074 ± 0.024 cd	***
ANOVA[b]	**	*	ns	*	ns	
FDI						
Aug-Oct	1.859 ± 0.201 c B	4.558 ± 0.575 a A	2.698 ± 0.153 b B	2.874 ± 0.713 b B	4.158 ± 0.571 a B	***
Nov-Jan	2.001 ± 0.046 bc B	3.243 ± 0.258 a B	1.917 ± 0.077 c C	1.851 ± 0.274 c C	2.032 ± 0.045 b BC	***
Feb-Apr	1.706 ± 0.220 ab B	1.827 ± 0.077 b C	1.961 ± 0.172 ab C	2.109 ± 0.277 a C	1.949 ± 0.116 ab C	*
May-Jul	2.472 ± 0.489 c A	2.980 ± 0.338 c B	3.855 ± 0.610 c A	7.544 ± 1.103 b A	14.23 ± 4.103 a A	***
ANOVA[b]	**	***	***	***	***	

Values in each colum ([b]ANOVA in capital letters) and row ([a]ANOVA in small letters) followed by the same letter do not differ significantly at p <0.05. (ns, not significant, * p <0.05, ** p <0.01, *** p <0.001). Aug-Oct, Nov-Jan, Feb-Apr, May-Jul indicate exposure periods; [a] ANOVA among exposure sites; [b] ANOVA among exposure periods.

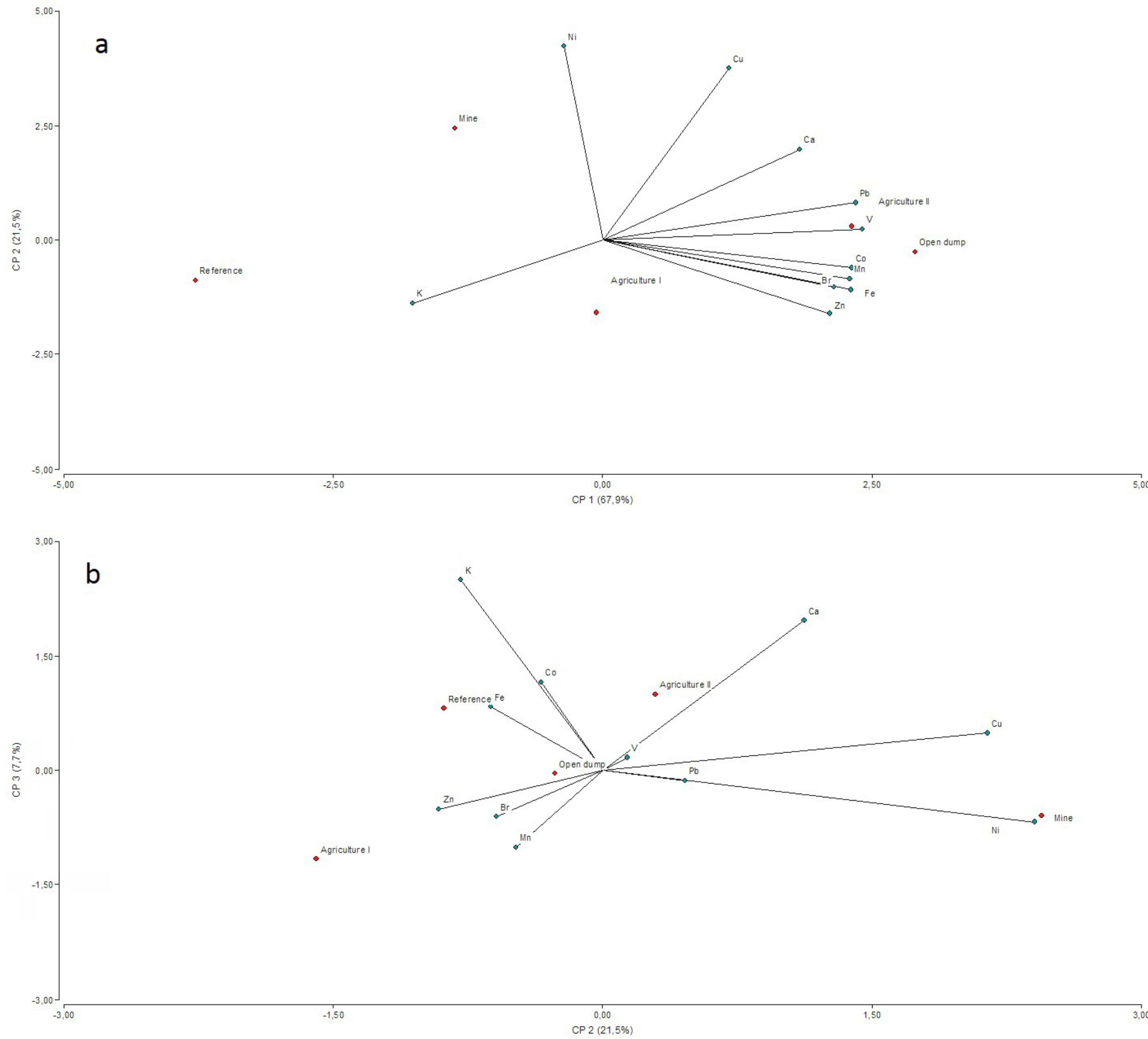

Figure 3: Biplot based on the first and second components (a) and the second and third components (b) of the Principal Component Analysis for trace elements measured in *T. capillaris*, using exposure areas (reference, agriculture-I, uranium mine, agriculture-II and open dump) as the classification criteria.

Table 4: Eigenvectors obtained by principal component analysis of trace element content in *T. capillaris*.

Variables	***T. capillaris***		
	Component 1	**Component 2**	**Component 3**
K	-0,27	-0,21	0,66
Ca	0,28	0,3	0,51
V	0,36	0,04	0,04
Mn	0,34	-0,13	-0,26
Fe	0,35	-0,16	0,22
Co	0,35	-0,09	0,30
Ni	-0,05	0,63	-0,18
Cu	0,18	0,56	0,13
Zn	**0**,32	-0,24	-0,14
Br	0,32	-0,16	-0,16
Pb	0,35	0,12	-0,03
Eigenvalues	**7.47**	**2.36**	**0.84**
–Cumulative proportion (%)	**0.68**	**0.89**	**0.97**

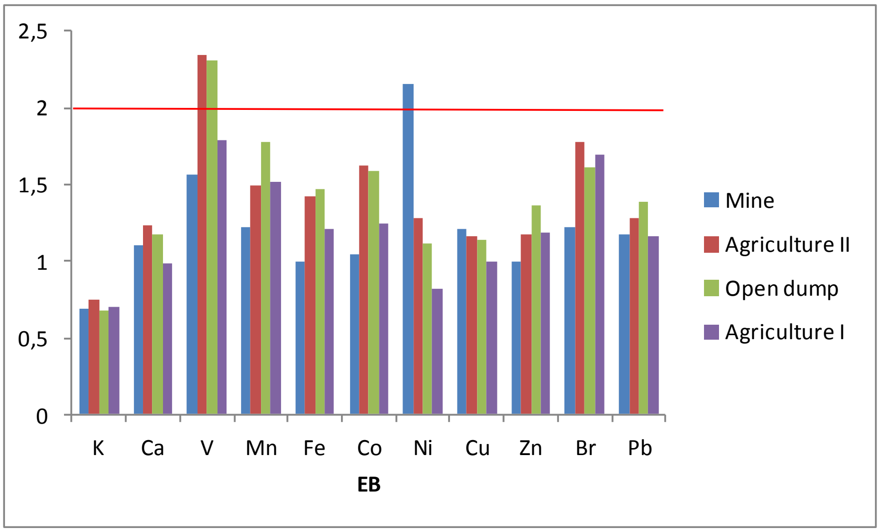

Figure 4: Exposed to basal ratios (EB) of trace metals measured in *T. capillaris* corresponding to different exposure areas (reference, agriculture, uranium mine, agriculture II and open dump). Values above the line of the y-axis (2) indicate severe enrichment of the metal in the biomonitor.

as NO_3^- and NH_4^+ [47], which are common in emissions from trash burning from either the biomass source or the fuel used [50-51]. In addition, unfavorable winter conditions such as low temperatures, little rainfall and low irradiation were related to the reduced content of chlorophyll in the plant (Figure 2a). This has also been reported in previous studies with *T. capillaris* [34] that point out that the chlorophyll content was affected by unfavorable weather conditions (drought and low temperature), which was accompanied by an increase of air pollutants. The membrane degradation parameters were most affected by anthropic activities related to mining and burning of waste, which may be a consequence of the release of oxidizing compounds and particulate matter enriched in trace metals, as reported in other studies [22; 24]. In addition, a biomonitoring study using the lichen *R. celastri* in a wide region of the province of Córdoba revealed a relation between high altitude and membrane lipid peroxidation in the biomonitor [42].

High sulfur content values were found at the open dump sites, which was related to the fuels used as well as to the characteristics of the burned biomass, as has been mentioned in other studies [51-53]. In a similar way, degradation of chlorophyll-a as a result of an increase in chlorophyll-b was observed at open dump sites; as mentioned above, this might have been related to oxidant emissions from burning of waste. Furthermore, the degradation of chlorophyll-a increased in the winter period due to unfavorable weather conditions and an increase in air pollutants as a result of domestic and industrial heating [34; 54].

The lowest water content in the leaves was detected at the former mine, as a result of both the pollutants that remained in the area and the elevated altitude. Moreover, the highest values of the DW/FW ratio corresponded to the exposure period that was characterized by higher temperatures and radiation, as previously reported by Pignata et al. [42].

Finally, the highest global physiological damage in the biomonitor was observed at the sites related to waste burning and mining activities. With regard to the open rubbish dump sites, numerous studies have reported that toxic pollutant emissions to the environment depend on characteristics of the waste and fuel [51; 55], whereas mining activities have revealed emissions of toxic heavy metals affecting vegetation and humans [22-24; 56-57]. Moreover, the winter period was identified as the one with the most physiological damage as a result of low temperatures, drought conditions and higher pollutant concentrations due to the increase in household fuel combustion.

4.2 Accumulation of nutrients and trace metals

Trace element accumulation in the biomonitor showed relationships among V, Mn, Fe, Co, Zn, Br and Pb and the open dump and agriculture-I sites. Practices such as open rubbish dumping have been previously mentioned as heavy metal sources [51; 55]. Regarding agricultural activities, numerous studies have pointed to the application of fungicides, pesticides and fertilizers as sources of heavy metals in soils and atmospheric particulate matter [36; 40; 58]. In fact, according to Shomar [59], pesticides are an important source of elements such as Al, Zn, Mn, Br, Cu, Ba, Cd, Co, Cr, Fe, Ni, Pb, Sc, and Sr, while phosphate fertilizers are a potential source of Cd, Zn, Cr, Cu, and Pb [60].

In the present study, the Cu and Ni contents came from the same emission source and were associated with the former uranium mine sites, with enrichment in nickel being observed as a result of mining activity due its multi-metallic characteristics. With regard to K and Ca, these macronutrients reflected the good physiological conditions of the biomonitor.

Finally, with respect to exposure periods, the climatic conditions of drought and low temperature resulted in the greatest physiological damage to the biomonitor. In contrast, the trace element accumulations showed the open-cast mine and open waste sites to be the main emission sources. Furthermore, this study verified the suitability of *Tillandsia capillaris* (Ruiz & Pav.) form *capillaris* as a trace element bioindicator for air quality in agricultural regions and mining areas, in addition to industrial and urban areas. Our results have revealed the necessity of avoiding open waste burning and the need for remediation of disused mining sites, due to toxic emissions affecting the environment and human health.

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