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## Biomonitoring of airborne fluoride and polycyclic aromatic hydrocarbons in industrial areas of Cordoba, Argentina, using standardized grass cultures of *Lolium multiflorum*

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### ABSTRACT

A biomonitoring study was performed employing standardized grass cultures. Plants of *Lolium multiflorum* were exposed at 4 industrial sites over three-month periods in two seasons (dry and rainy) and the biomass produced was used for subsequent measurements of fluoride, polycyclic aromatic hydrocarbons (phenanthrene, anthracene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene and benzo[*g,h,i*]perylene), total chlorophyll, malondialdehyde, water, and sulfur content. The total content of polycyclic aromatic hydrocarbons (PAHs) revealed seasonal variations, with the highest values corresponding to the dry season, although this species showed a high retention capacity of PAHs during rainy season. In addition, sampling sites with high vehicular traffic and metal–mechanical industries were associated with the highest content of PAHs. Furthermore, physiological degradation associated with anthropogenic activities in the sampling sites was observed. Fluoride content in the biomonitor was associated with the production and use of cement, which was higher in the dry season.

**Keywords:** *Lolium multiflorum*, PAHs, fluoride, biomonitoring, physiological response



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### 1. Introduction

As a result of the urbanization process and industrialization, the pollutant emissions have substantially increased in developing countries, thereby deteriorating the environmental quality in many agglomerations and industrial areas of South American countries (Klumpp et al., 1996; Romero et al., 2005). Emissions from industrial processes, such as brick and ceramic factories, phosphate fertilizer plants and aluminum smelters, release fluorides and polycyclic aromatic hydrocarbons (PAHs), among other pollutants, to the environment. Fluoride compounds constitute one of the most important phytotoxic air pollutants, being between 1 and 3 orders of magnitude more toxic than other common pollutants, (Weinstein and Davison, 2004) with the fluoride concentration in the air varying according to industrial emissions, weather factors and topography (Divan Junior et al., 2008). Moreover, visible damage to leaves by fluoride has been reported (Weinstein and Davison, 2003), but also changes in the physiological responses of plants, including composition and function of cell membranes, photosynthesis, respiration and metabolism of carbohydrates (Fornasiero, 2001; Divan et al., 2007; Sandrin et al., 2008).

PAHs, on the other hand, are a class of persistent organic pollutants, ubiquitous in the environment, which are the product

of thermal decomposition during the incomplete combustion of organic materials and the geochemical formation of fossil fuels. Moreover, some compounds of PAHs may pose a threat to humans because of their mutagenic, carcinogenic, teratogenic and immunosuppressive properties (Lehndorff and Schwark, 2004). PAHs are removed from the atmosphere both in the vapor–phase and condensed form, adsorbed to aerosol particles, and deposited on water, soil and plant foliage (Orecchio, 2007). The vegetation canopy is the first surface available to the major atmospheric pollutants when they are deposited on terrestrial ecosystems (Murray, 1982), with deposition of pollutants on the plant foliage being a function of the air concentrations, while the accumulation in the vegetation depends on the particular properties of the contaminant as well as on the properties of the accumulating surface (Riederer, 1990). Consequently, biomonitoring employing vegetation is an effective alternative for detecting environmental hazards and has been employed in many studies as well as in biomonitoring networks (Klumpp et al., 2004; Nobel et al., 2005; Abril et al., 2014). Among species employed in biomonitoring studies the use of lichens (Protano et al., 2014), mosses (Qarri et al., 2014), bromeliads (Rodríguez et al., 2010) as well as tropical trees (Sant'Anna–Santos et al., 2014) have been widely studied. In addition, biomonitoring of pollutants is receiving increasing attention as an alternative to conventional methods, particularly in areas where complex and expensive air sampling devices are

unavailable (Sun et al., 2010). One of the advantages of biomonitoring over instrumental monitoring is that it can provide abundant reliable information of the impact of airborne pollutants on the physiological processes (De Temmerman et al., 2004). Plants can take up fluoride from the air directly via the stomata or via deposition on the foliage surfaces (Rodriguez et al., 2012) while PAH deposition depends on gas-to-particle conversion (Simonich and Hites, 1994). Furthermore, plants may also incorporate fluoride and PAHs from soil by absorption through the roots (Wang et al., 2012). However in general these concentrations are often negligible because detoxification processes taking place in the roots (Binet et al., 2000; Rey-Asensio and Carballeira, 2007).

Biomonitoring is a particularly effective tool in developing countries to avoid the high cost of instrumental monitoring (Klumpp et al., 1996; Romero et al., 2005), and it has also been recommended by European legislation for assessing the impact of PAHs on ecosystems (EU, 2004). The use of biomonitor species with a standardized response has become increasingly important as a consequence of the good results obtained and their high reproducibility (Klumpp et al., 2004). The standardization of methods is an essential step in order to obtain comparable results across different sites. Thus, the standardized ryegrass *Lolium multiflorum* (VDI, 2003) has been used for the monitoring of the trace elements (Klumpp et al., 2009); polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans, PAHs, polychlorinated biphenyls (PCBs) (Radermacher and Krause, 2002); heavy metals (Romano Sant'Anna et al., 2004; Sandrin et al., 2004); sulfur (Romano Sant'Anna et al., 2004) and fluoride (Klumpp et al., 1994;

Klumpp et al., 1996; Franzaring et al., 2006; Franzaring et al., 2007).

Thus, the aims of this research were: (i) to verify the biomonitoring ability of *L. multiflorum* for fluoride and PAHs emitted by industrial sources, (ii) to evaluate the relationships between the accumulation of fluoride, PAHs and the physiological response of grass cultures in the vicinity of industries, and (iii) to study the seasonal variations of PAHs and fluoride and their relationship to different emission sources.

## 2. Materials and Methods

### 2.1. Study area and cultivation of standardized grass cultures

The study region included four areas in Cordoba province located in central Argentina, characterized by the presence of industries (Figure 1). The exposure area included the city center (CTR) and two areas near the city of Cordoba (1.5 million inhabitants): Ferreyra neighborhood (FER) of Cordoba City, an industrial sector with metallurgical and metal-mechanical industries, a cement plant in the locality of Yocsina (YOC) at 18 km southwest from Cordoba City, and a fourth area situated in the city of Monte Cristo (MOC) 25 km west of Cordoba City, characterized by a granite floor production plant and a plant of liquid fuel distribution. In addition, grass cultures (N=3) were maintained under controlled conditions in the greenhouse during the sampling periods in order to be able to consider them as a baseline material and used as reference controls. Table 1 shows the environmental characteristics of the exposure sites.

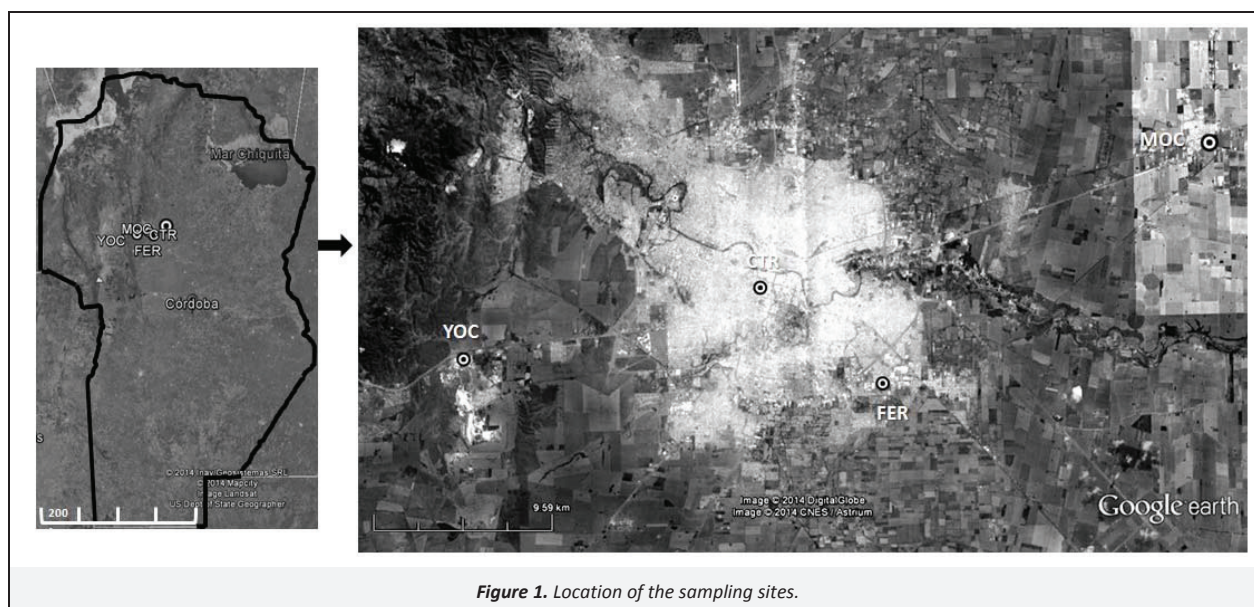


Figure 1. Location of the sampling sites.

Table 1. Exposure sites and their environmental characterization

Exposure Sites	Main Pollutants Emission Sources	Altitude (m.a.s.l.)	Inhabitants (INDEC Surveys, 2001 and 2010)	Location Latitude/Longitude	Total PAHs (ng passive air sampler) <sup>c</sup>	
					Dry Season (21/03–21/06)	Rainy Season (1/09–1/12)
Center (CTR)	Vehicular traffic	410	28 949 <sup>a</sup>	31°24'46.9" S/ 64°11'53.7" W	6 390	3 793
Ferreyra (FER)	Metal and mechanical industries	408	3 613 <sup>a</sup>	31°27'39.1" S/ 64°7'46.7" W	10 574	7 881
Yocsina (YOC)	Cement industry (using 25% alternative fuels)	580	1 336 <sup>a</sup>	31°26'40.2" S/ 64°22'2.8" W	8 364	1 305
Montecristo (MOC)	Production of granite floors Liquid fuel distribution	350	9 254 <sup>b</sup>	31°20'47.9" S/ 63°56'23.7" W	3 690	2 609

Note: INDEC, Instituto Nacional de Estadísticas y Censos, <sup>a</sup> 2001, <sup>b</sup> 2010; <sup>c</sup> passive air samplers original data reprinted from Wannaz et al. (2013)

In this study, two samplings campaigns were carried out, one during the dry season (from March 21<sup>st</sup> to June 21<sup>st</sup>) and the other during the rainy season (from September 1<sup>st</sup> to December 1<sup>st</sup>), whose climatic characteristics have been reported in Wannaz et al. (2013). Briefly, the dry season was characterized by an average temperature of 15.8 °C and cumulative rainfall of 57.1 mm, while the rainy season had an average temperature of 19.4 °C and 252 mm of cumulative rainfall.

In the present study we used Italian ryegrass [*Lolium multiflorum* (LAM) ssp. *italicum* cv. Lema] biomonitors, with the cultures being grown in a greenhouse and strictly adhering to the standard protocol of the Association of German Engineers VDI (2003). Briefly, 0.6 g of grass seeds were sown in plastic pots (A=15.4 cm<sup>2</sup>) filled with non-fertilized cultivation substrate, and plants were trimmed with a pair of scissors to a height of 4 cm three times before exposure. Cutting back the grass and providing a regular supply of fertilizers ensured a vigorous plant tillering and growth. Plants were fertilized twice during pre-culture with an NPK-fertilizer made of analytical-grade chemicals, with plants being watered in the field automatically using glass fiber wicks (5 mm diameter) and a water reservoir of deionized water of 5 L. Grass cultures were cut back to 4 cm and fertilized again immediately prior to exposure to the sampling sites. In the field, the water reservoir only had to be replenished when the weather became hot and sunny for a couple of days.

## 2.2. Exposure methods

As mentioned above, two sampling campaigns corresponding to the dry (21/03/2011 to 21/06/2011, autumn) and rainy (01/09/2011 to 1/12/2011, spring) seasons were performed. During the experimental period for each season, grass cultures were exposed for three consecutive periods of 4 weeks each according to VDI (2003). Then, after each exposure period, the cultures were sampled and replaced by new ones.

## 2.3. Chemical determinations

Three replicates were measured at each exposure area after an exposure period of 4 weeks, making a total of 9 determinations over 12 weeks of exposure. All concentrations were expressed on a dry weight basis.

**Extraction, cleanup and analysis of PAHs.** The reagents, cleaning and analytical procedures have been previously detailed in Wannaz et al. (2013). Briefly, PAH standard solutions (EPA 525 PAH Mix B, SUPELCO) were employed as calibrants following the external calibration method (0.2–50 ng mL<sup>-1</sup>). These solutions contained acenaphthylene (ACE), phenanthrene (PHE), anthracene (ANT), pyrene (PYR), benzo[*a*]anthracene (BaA), chrysene (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenzo [*a,h*]anthracene (DBA), indeno[1,2,3-*c,d*]pyrene (IPY) and benzo[*g,h,i*]perylene (BPE). However, it is important to note that ACE was not measured because it has no fluorescence, while IPY was not determined since it elutes between DBA and BPE, with the quantification of this compound not being good. The extraction and quantification procedure was performed according to the miniaturized method of Sanz-Landaluze et al. (2010). Briefly, 300 mg of plant material was placed in the extraction cell with 2 mL of *n*-hexane and acetone (1:1, v/v) and then immersed in an ultrasonic water bath for 30 s employing an ultrasonic probe with a 3 mm titanium microtip. Subsequently, extracts were filtered with Sartorius filters (0.22 µm, Gottingen, Germany) and evaporated by a nitrogen flow. Then, the residue was dissolved with 0.4 mL of acetonitrile and water (6:4, v/v) and the sample was analyzed using a HPLC Perkin Elmer Series 200 (Perkin Elmer, Norwalk, CT, USA) equipped with a programmable fluorescence detector. The qualitative and quantitative determinations were carried out using a C18 column (Vydac 201TP, USA) at a

flow rate of 0.3 mL min<sup>-1</sup> with a elution gradient program started at acetonitrile:water (60:40, v/v) for 18 min, then acetonitrile 100%, and after 3 min changing back to the initial phase. Reliability of the analytical procedure was verified measuring a certified reference plant material (IAEA-140 OC, organochlorine compounds and petroleum hydrocarbons) repeatedly every 10 measurements, with a recovery rate between 66% and 103% (Table 2).

**Table 2.** Concentration of PAHs from the extract of the Certified Reference Material IAEA-140 OC (seaweed) and percentage recoveries [Original data reprinted from Wannaz et al. (2013)]

PAH	Certified Value (ng g <sup>-1</sup> )	Values Obtained (ng g <sup>-1</sup> ) n=4	Recovery (%)
PHE	76	62.4±13.8	86
ANT	14	10.2±2.5	73
PYR	67	69.0±7.2	103
BaA	25	23.8±4.5	95
CHY	40	31.5±7.4	79
BkF	19	12.5±6.2	66
BaP	20	17.7±6.1	89
BPE	20	19.8±4.3	99

**Fluoride analysis in *L. multiflorum*.** The fluoride analysis in leaves of *L. multiflorum* followed the method described in VDI (1998) and was based on an alkali melt, with fluoride levels being measured using an ion-sensitive electrode (ISE F 800 DIN, WTW Weilheim, Germany) coupled to an ionmeter (Inolab pH/Ion 735 WTW Weilheim, Germany). Further details on this analysis are described in Franzaring et al. (2006). The calibration was made using NaF standard solutions, and to assure the quality of the fluoride analysis a certified standard was used.

**Physiological determinations in *L. multiflorum*.** Quantification procedures for chlorophyll-*a* (Chl-*a*), chlorophyll-*b* (Chl-*b*), malondialdehyde (MDA), sulfur content (S) and dry weight/fresh weight ratio (DW/FW) consisted in measurements being performed as previously described by Pignata et al. (2002). Physiological measurements were performed using standard calibration curves, as well as specific molar extinction coefficients for each determination. All concentrations were expressed using a dry weight basis (g<sup>-1</sup> DW).

## 2.4. Evaluation of PAH biomonitoring ability of *L. multiflorum*

A comparison was conducted between *L. multiflorum* and the biomonitor *Tillandsia capillaris* Ruiz and Pav. form *capillaris* with the purpose of assessing the availability of PAHs accumulation of the species employed in this study. It should be noted that *T. capillaris* was exposed simultaneously in the study area and these results have been reported by Wannaz et al. (2013). The epiphyte *T. capillaris* has been widely employed in biomonitoring studies of heavy metals and PAHs (Brighigna et al., 2002; de Souza Pereira et al., 2007; Rodriguez et al., 2010; Wannaz et al., 2013). Detailed information about the collection and exposure of plant material are described in Wannaz et al. (2013). Briefly, plants of *T. capillaris* were collected in an unpolluted site in Cordoba Province (Intiyaco), which were subsequently exposed in net bags at 3 m above ground level in the sampling sites for 12 weeks in each exposure period (dry and rainy). Then, the concentration of PAHs in the biomonitors was analyzed as mentioned in Section 2.3.

## 2.5. Statistical analysis

Physiological parameters, fluoride and PAH concentrations for each monitoring season were submitted to an 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).

A Principal Component Analysis (PCA) was performed using the sampling sites as the classification criteria in order to assess the relationship among them and the accumulation of PAHs in the biomonitor. This analysis was done with the purpose of reducing the dimensionality of the data matrix, in order to avoid redundancy and to highlight relationships. It should be noted that the assumptions of PCA were met (with continuity of the variables and the number of elements observed being greater than the number of original variables).

The bioaccumulation of metals and PAHs in biomonitors has been evaluated in several studies (Bergamaschi et al., 2007; Sorbo et al., 2008; Guidotti et al. 2009; Protano et al., 2014) based on the exposed–to–control (EC) ratio introduced by Frati et al. (2005). In this study a modified ratio of exposed to–control–ratio (EC) for metals, called the EB ratio (exposed to–basal–ratio) for PAHs was employed according to Wannaz et al. (2013). EB is the ratio between the PAH concentrations in exposed samples with those of

baseline samples. According to this ratio values are interpreted based on the following criteria (Fratti et al., 2005): severe loss–based EB=0.00–0.25, somewhat loss–based EB=0.25–0.75, normal EB=0.75–1.25, accumulation EB=1.25–1.75, and severe accumulation EB>1.75. All analyses were performed using Microsoft Excel software 2010 for Windows™ and InfoStat software, Version 2012.

### 3. Results and Discussion

#### 3.1. Polycyclic aromatic hydrocarbons

The compounds were grouped into classes according to the number of aromatic rings present in their structures, thus compounds with low molecular weights (LMW) are those with two or three aromatic rings, and compounds with high molecular weights (HMW) have four or more aromatic rings. This classification was performed with the purpose of establishing a relationship between the volatility of the compounds and the monitoring sites. Table 3 shows the mean concentrations and ANOVA results of individual PAH, LMW, HMW and total PAHs accumulated in the leaves of *L. multiflorum*, corresponding to the dry and rainy seasons, respectively.

**Table 3.** Concentrations of PAHs and the exposed to basal ratio (EB ratio) in *Lolium multiflorum* at different sites in dry and rainy season

PAHs (ng g <sup>-1</sup> DW)	Dry Season Sampling Sites					ANOVA
	Basal	CTR	FER	YOC	MOC	
PHE	5.38	89.5 (1.66)	100 (1.86)	57.9 (1.07)	84.6 (1.57)	ns
ANT	4.71	8.36 (1.77)	8.89 (1.89)	6.26 (1.33)	5.68 (1.21)	ns
PYR	1.55	31.5 (2.02)	18.6 (1.20)	33.6 (2.16)	25.0 (1.61)	ns
BaA	nd	5.88 (2.76)	5.37 (2.52)	2.67 (1.25)	4.28 (2.01)	ns
CHR	nd	11.9 (6.75) a	9.45 (5.36) a	4.76 (2.70) b	3.10 (1.76) b	$p < 0.01$
BbF	nd	9.37 (1.66)	15.7 (2.78)	9.66 (1.71)	9.07 (1.61)	ns
BkF	nd	0.47 (2.14)	0.21 (0.97)	0.22 (1.01)	0.25 (1.14)	ns
BaP	nd	1.45 (4.76)	0.64 (2.09)	0.63 (2.08)	0.64 (2.11)	ns
DBA	nd	0.80 (1.75) c	0.95 (2.09) b	1.64 (3.59) a	1.10 (2.40) b	$p < 0.01$
BPE	nd	1.62 (2.23)	4.91 (6.76)	5.02 (6.90)	3.02 (4.16)	ns
Total PAHs	11.65	453 ab	464 a	348 c	402 bc	$p < 0.1$
ΣLMW	11.65	285	318	185	265	
ΣHMW	nd	168	146	162	138	
Rainy Season						
PHE	4.26	56.6 (1.33)	75.6 (1.77)	68.1 (1.60)	66.0 (1.55)	ns
ANT	5.90	6.00 (1.02)	6.57 (1.11)	6.34 (1.07)	6.21 (1.05)	ns
PYR	2.37	38.7 (1.63)	44.3 (1.86)	26.4 (1.11)	29.4 (1.24)	ns
BaA	nd	4.73 (1.98)	5.37 (2.25)	6.95 (2.91)	4.86 (2.04)	ns
CHR	nd	4.56 (1.39)	6.31 (1.92)	6.50 (1.98)	4.63 (1.40)	ns
BbF	nd	10.8 (1.66) ab	11.9 (1.83) a	4.38 (0.67) b	6.16 (0.95) ab	$p < 0.1$
BkF	nd	1.32 (2.24)	0.78 (1.33)	0.76 (1.29)	0.86 (1.45)	ns
BaP	nd	0.51 (0.98)	0.56 (1.08)	0.61 (1.18)	0.54 (1.05)	ns
DBA	nd	2.18 (2.20)	5.79 (5.84)	9.12 (9.20)	2.18 (2.20)	ns
BPE	nd	4.52 (3.26)	4.41 (3.18)	4.06 (2.93)	4.12 (2.97)	ns
Total PAHs	12.53	375 b	459 a	367 c	362 c	$p < 0.01$
ΣLMW	12.53	188	240	217	210	
ΣHMW	nd	188	219	150	151	

Note: Values in each row followed by the same letter do not differ significantly. (ns: not significant)

ΣLMW: sum of compounds with low molecular weights, ΣHMW: sum of compounds with high molecular weights, nd: not detected

The mean concentrations of total PAHs in the leaves of *L. multiflorum* ranged from 347.92 (YOC sampling site) to 463.98 ng g<sup>-1</sup> DW (FER sampling site), and for the baseline material a concentration of 12 ng g<sup>-1</sup> DW was found (Table 3). It is important to note that only concentrations of PHE, ANT and PYR were found in the baseline material, with no high-molecular-weight compounds being detected in *L. multiflorum*. In addition, our results were lower than those reported in *L. multiflorum* in the vicinity of a large industrial complex in Brazil (Rinaldi et al., 2012), and those reported by Rodriguez et al. (2010) using the biomonitor *T. capillaris* exposed to different levels of vehicular traffic in Germany, as well as those reported by Protano et al. (2014) using the lichen *Pseudovernia furfuracea* nearby to a solid-waste landfill in central Italy. However, the PAH values reported in the present study were similar to the results of Dan-Badjo et al. (2007, 2008), who employed *L. multiflorum* at different distances from a road, as well as with values reported by Klumpp et al. (1996) using curly kale as a biomonitor of PAHs in many European countries and also similar with the values informed by Guidotti et al. (2009) employing *P. furfuracea* as biomonitor at different traffic densities in Italy.

With regard to the different seasonal exposures, our results showed that PHE and PYR were the most abundant PAHs in both sampling seasons (Figure 2), as has also been reported by Dan-Badjo et al. (2008) using *L. multiflorum*. Moreover, our results showed that in general the total concentrations of PAHs were higher in the biomonitors exposed during the dry season, with the values of  $\Sigma$ LMW results being consistent with those reported by using the biomonitor *T. capillaris* and passive air sampling (Wannaz et al., 2013), and instrumental monitoring (Amador-Munoz et al., 2010; Masih et al., 2012; Amador-Munoz et al., 2013). Nevertheless, some higher values of  $\Sigma$ HMW in this study were found in the rainy season, especially for site FER. The fact that more PAHs have been found in the dry season in numerous studies has been attributed to rainfall, which produced a decrease in the content of PAHs through the washing of the plants (Rinaldi et al., 2012) and also to the removal of pollutants from the atmosphere (Tham et al., 2008). In addition, in the present study the dry season coincides with lower temperatures, with a higher concentration of PAHs in the atmosphere having been reported at low temperatures as a result of domestic and industrial carbon burning (Amador-Munoz et al., 2010; Amador-Munoz et al., 2013; Cabuk et al., 2014). It is also important to note, conversely, that in the rainy season temperatures and global irradiation were higher in our investigation, and it has been previously reported that PAHs undergo photodegradation, leading to a decrease in their

concentrations in the atmosphere (Baek et al., 1991; Chetwittayachan et al., 2002; Amador Munoz et al., 2013). Furthermore, the EB ratios showed a severe PAH accumulation (>1.75) in many compounds, which were also higher in the dry season in comparison with the rainy season. Moreover, in the dry season the sites CTR and FER showed the greater number of PAHs with severe accumulations (CTR site: ANT, PYR, BaA, CHR, BkF, BaP, DBA and BPE; FER site: PHE, ANT, BaA, BbF, CHR, BaP, DBA and BPE), whereas in the wet season FER revealed the highest number of PAHs with severe accumulation (PHE, PYR, BaA, CHR, BbF, DBA and BPE). These results are consistent with those observed by Wannaz et al. (2013) mainly due to the characteristics of these sites, which have high vehicular traffic and metal-mechanical activities, whose increased emissions of PAHs which are ultimately reflected in the biomonitors.

Although the comparison between sampling sites for many individual PAHs showed no significant differences, comparing the total content of PAHs between the dry and the wet seasons did reveal significant results (Table 3). In fact, for both the dry and rainy seasons the highest values of total PAHs were found at the FER site followed by the CTR site, which also showed elevated levels of PAHs in passive air samplers (Wannaz et al., 2013). Regarding to that FER site was characterized by metal-mechanical industries and heavy vehicular traffic, while the CTR site was characterized by high vehicular traffic (cars and buses).

A principal component analysis (PCA) was conducted in order to identify the PAHs associated with the study sites and using different sampling sites as the classification criteria, similar results were obtained to those mentioned above, since a positive association was indicated between the FER and CTR sites with most of the PAHs accumulated in the biomonitor (Table 4 and Figure 3). As mentioned previously, both sites presented vehicular traffic which was an important source of emission of PAHs. Furthermore, in relation to these sources, our results revealed that most of the PAHs analyzed in the biomonitor (BbF, ANT, CHR, BaA and PYR) contributed to the first component, which may indicate a common emission source of these compounds. On the other hand, the variables BaP, BkF and BPE contributed to the second component, of which the first two were negatively associated with the component, there by showing an inverse relationship and possibly indicating that two emission sources were related to this component. Finally, with regard to the third component, this was associated with DBA but negatively associated with PHE, indicating two possible sources of emission of these compounds.

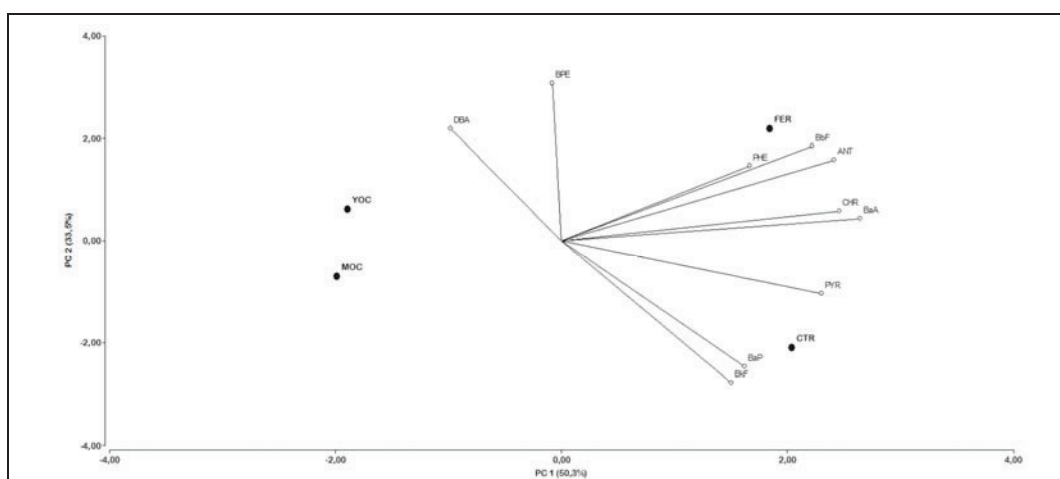


Figure 2. Bi-plot based on the first two components of the principal components analysis for PAH concentrations in *L. multiflorum*, using different sites as the classification criterion.

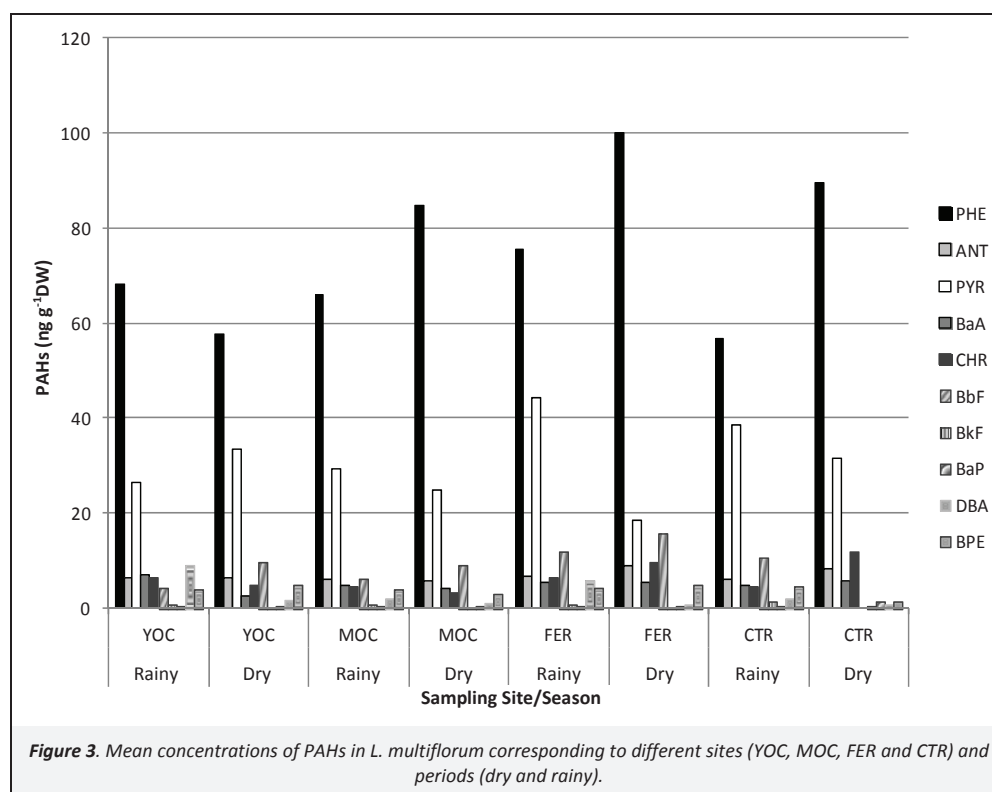


Figure 3. Mean concentrations of PAHs in *L. multiflorum* corresponding to different sites (YOC, MOC, FER and CTR) and periods (dry and rainy).

Table 4. Eigenvectors obtained by principal component analysis of the PAHs measured in *Lolium multiflorum* in both seasons

Variable	Component 1	Component 2	Component 3
PHE	0.27	0.24	-0.54
ANT	0.39	0.26	0.03
PYR	0.38	-0.17	0.35
BaA	0.43	0.07	-0.18
CHR	0.40	0.09	0.32
BbF	0.36	0.30	-0.15
BkF	0.24	-0.45	0.08
BaP	0.26	-0.40	0.26
DBA	-0.16	0.36	0.52
BPE	-0.01	0.50	0.32
Eigenvalues	5.03	3.35	1.62
Accumulated variance (%)	50	84	100

Figure 4 shows the comparison between the PAHs accumulation in *L. multiflorum* and *Tillandsia capillaris*. In the rainy season it was observed that *L. multiflorum* showed higher PAHs values than *T. capillaris*, indicating a higher retention of these compounds during rain events by *L. multiflorum*. In contrast, in the dry season a higher PAH content was observed at the MOC and CTR sites in *L. multiflorum*, and the FER site in *T. capillaris*. In addition, it is important to highlight that a similar relationship between low and high molecular weight PAHs was found among species and exposure sites.

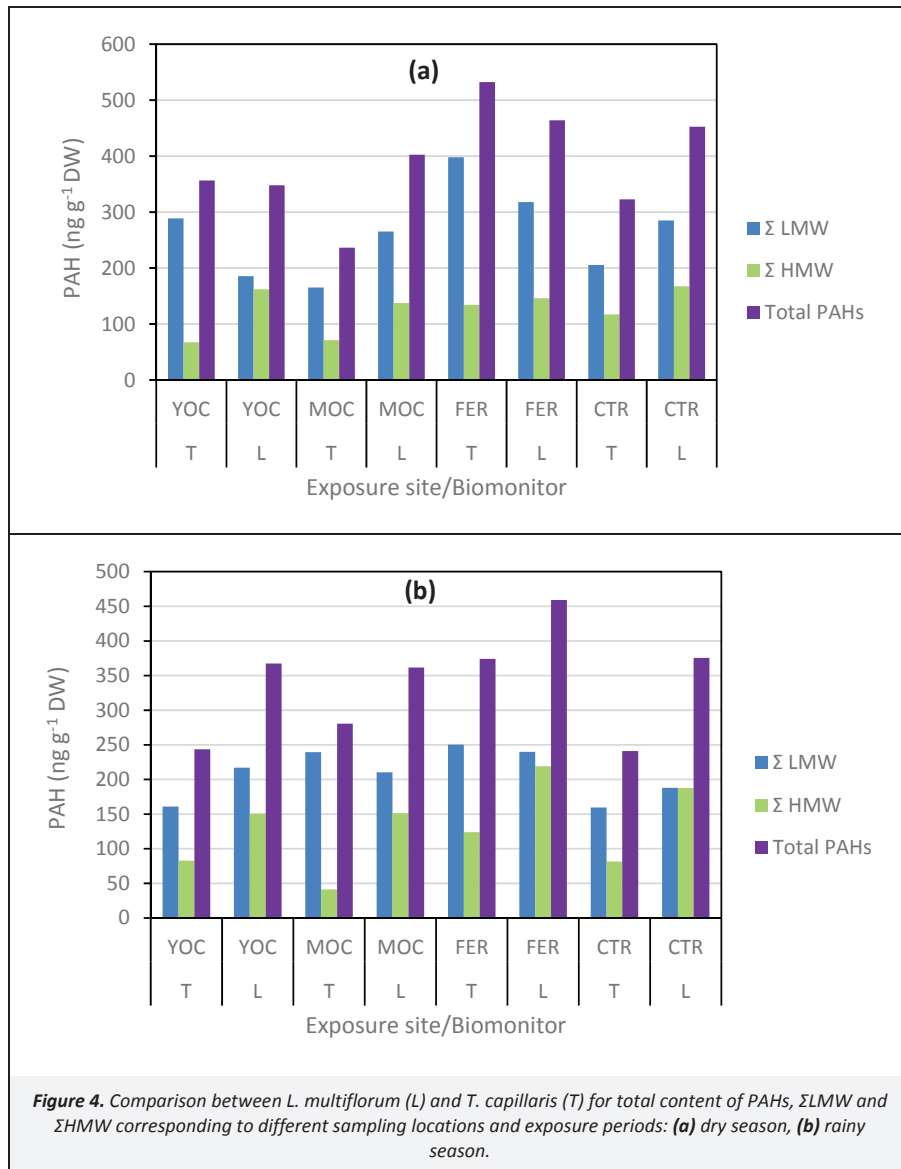
### 3.2. Fluoride biomonitoring and physiological response using *L. multiflorum*

Table 5 shows the results of total chlorophyll, Chl-b/Chl-a ratio, malondialdehyde content, water status, sulfur and fluoride content in leaves of *L. multiflorum*, corresponding to different

sampling sites and seasons, which showed significant differences for almost all the parameters evaluated in the sampling sites. The total chlorophyll content showed the highest values at the FER and YOC sites in the dry season, which may respond to a possible fertilization effect of particulate material rich in metal ions such as Fe in the case of metal-mechanical industries and calcium in the case of the cement industry. In contrast, in the rainy season a marked increase in the photosynthetic pigments was observed, which was mainly due to the higher temperature in this season promoting greater plant growth accompanied by a chlorophyll increase. In the dry season, the Chl-b/Chl-a ratio showed significantly greater values for the CTR site, which indicate a degradation of chlorophyll-a over chlorophyll b, due to air pollutants from vehicular traffic (Arb and Brunold, 1990; Saitanis et al., 2001; Rodriguez et al., 2007).

Regards to the MDA parameter, which indicates degradation of cell membranes, the highest values were found at the MOC site followed by the FER site in both sampling periods, indicating that human activities at both sites were largely responsible for membrane degradation in plants. In addition, the biomonitoring studies revealed an increase in the oxidation of membrane lipids due to other industrial pollutant emissions (Pignata et al., 2007; Rodriguez et al., 2011).

The DW/FW parameter indicates the water content of biomonitors. Many biomonitoring studies have mentioned a direct relationship between the accumulation of contaminants and a decrease in water content of biomonitors (Pignata et al., 2002; Wannaz and Pignata, 2006; Bermudez et al., 2009), with this parameter being a good indicator of physiological damage. In this study, the highest values were observed in the samples at the MOC and FER sites, corresponding to the dry and rainy seasons respectively, which might be related to pollutant emissions associated with metal-mechanical activities, the production of granite floors and liquid fuel distribution.



**Figure 4.** Comparison between *L. multiflorum* (L) and *T. capillaris* (T) for total content of PAHs, ΣLMW and ΣHMW corresponding to different sampling locations and exposure periods: (a) dry season, (b) rainy season.

**Table 5.** Mean concentrations ± SD of total chlorophyll (Chl-a+b), Chl-b/Chl-a ratio, malondialdehyde (MDA), dry weight/fresh weight ratio (DW/FW), sulfur content and fluoride content (F<sup>-</sup>) measured in *Lolium multiflorum* corresponding to different sites and seasons

Season	Chemical Parameter	Sampling Sites					ANOVA
		Basal	CTR	FER	YOC	MOC	
Dry	Chl-a+b (mg g <sup>-1</sup> DW)	0.62±0.03 ab	0.51±0.09 b	0.85±0.10 a	0.87±0.19 a	0.67±0.16 ab	p<0.01
	Chl-b/Chl-a	0.42±0.01 b	0.56±0.05 a	0.40±0.04 b	0.40±0.04 b	0.41±0.06 b	p<0.001
	MDA (nmol g <sup>-1</sup> DW)	54.0±4.12 b	72.3±18.0 b	81.7±14.1 ab	69.4±11.2 b	100±24.4 a	p<0.1
	DW/FW	0.12±0.01 c	0.15±0.02 ab	0.15±0.02 abc	0.14±0.02 bc	0.17±0.02 a	p<0.1
	Sulfur (mg g <sup>-1</sup> DW)	2.18±0.07 b	4.08±0.79 a	2.36±0.44 b	2.17±0.11 b	2.00±0.21 b	p<0.001
	F <sup>-</sup> (µg g <sup>-1</sup> DW)	6.03±1.45 d	26.3±0.70 b	16.7±1.28 c	30.4±3.18 a	29.8±1.58 a	p<0.001
	Chl-a+b (mg g <sup>-1</sup> DW)	2.60±0.08 a	1.58±0.34 b	1.07±0.17 d	1.50±0.22 bc	1.17±0.35 cd	p<0.001
Rainy	Chl-b/Chl-a	0.38±0.01	0.37±0.05	0.38±0.06	0.38±0.03	0.37±0.02	ns
	MDA (nmol g <sup>-1</sup> DW)	47.1±4.83 c	62.9±9.21 bc	82.6±21.2 ab	65.0±17.9 bc	89.0±25.9 a	p<0.1
	DW/FW	0.12±0.01 c	0.16±0.03 c	0.31±0.12 a	0.18±0.04 bc	0.26±0.08 ab	p<0.01
	Sulfur (mg g <sup>-1</sup> DW)	1.64±0.46 c	2.86±0.48 b	3.46±0.53 a	3.31±0.03 ab	3.08±0.46 ab	p<0.01
	F <sup>-</sup> (µg g <sup>-1</sup> DW)	3.24±0.08 c	19.5±5.72 ab	16.9±0.59 b	14.8±1.34 b	22.9±6.65 a	p<0.001

Values in each row followed by the same letter do not differ significantly, ns: not significant

With respect to the sulfur content in the biomonitor, it was observed that the highest values corresponded to the CTR and FER sites for the dry and rainy seasons, respectively. These results may indicate combustion processes from vehicular traffic, since sulfur is an element commonly found in fuels and these sites are characterized by the presence of high vehicular traffic from due to buses and cars (CTR), and large vehicles such as trucks (FER).

The fluoride content in the biomonitor leaves was significantly higher at the MOC site in both sampling periods, while in the dry season higher values were also observed at the YOC site. The YOC site was characterized by the presence of a cement plant, although the emission of gaseous fluoride was unlikely to be due to Portland cement clinker burning resulting in excess CaO, which generates CaF<sub>2</sub>. However, particulate matter can be enriched in fluoride and reach the surrounding vegetation (U.S. EPA, 1971; WBCSD, 2012). Moreover, the use of calcium fluoride has also been reported for the mineralization of cement (Thuan, 2011). On the other hand, regarding the high levels of fluoride associated with the MOC site, it is important to note that this site was characterized by the production of granite floors, whose production process involved cement, so it is likely that the high levels observed in the biomonitor corresponded to deposition of particulate material enriched with fluoride from this cement. Finally, it is important to note that the fluoride content was higher in the periods coinciding with lower rainfall and temperatures, as we reported for the total content of PAHs in the biomonitor. Therefore, fluoride biomonitoring by *L. multiflorum* responds to climatic characteristics and should be used in periods with low rainfall.

#### 4. Conclusions

The concentrations of PAHs and fluoride measured in leaves of the biomonitor *L. multiflorum* showed seasonal differences, indicating that the rainy season, which in this study also coincided with higher temperatures and total irradiance promotes the removal of pollutants in the environment. In contrast, dry periods are characterized by low temperatures and hence an increase in domestic and industrial carbon burning and greater emissions of pollutants to the environment. In this study, the species *L. multiflorum* has proven to be more efficient in accumulating PAHs than *T. capillaris* during rainy periods. Moreover, *L. multiflorum* was able to detect different pollutant sources, in particular sites of vehicular traffic with a high content of PAHs, which was also reflected by the physiological status of the biomonitor. In contrast, the production of cement was revealed as the main emission source of fluorides.

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