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Accumulation of polycyclic aromatic hydrocarbons and trace elements in the bioindicator plants *Tillandsia capillaris* and *Lolium multiflorum* exposed at PM_{10} monitoring stations in Stuttgart (Germany)

J.H. Rodriguez [a], M.L. Pignata [a], A. Fangmeier [b], A. Klumpp [b,*]

[a] Multidisciplinary Institute of Plant Biology, Section Pollution and Bioindicators, Faculty of Physical and Natural Sciences, National University of Cordoba, Av. Vélez Sársfield 1611, X5016CGA Cordoba, Argentina

[b] Institute of Landscape and Plant Ecology (320), Plant Ecology and Ecotoxicology, Universität Hohenheim, August-von-Hartmann-Str. 3, 70599 Stuttgart, Germany

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ABSTRACT

The accumulation of polycyclic aromatic hydrocarbons (PAHs) in *Tillandsia capillaris* Ruiz and Pav. form *capillaris* and trace elements in *T. capillaris* and *Lolium multiflorum* (LAM) cv. Lema was assessed and evaluated in the city of Stuttgart, Germany. Several sites (urban, suburban and rural) categorized according to type and intensity of vehicular traffic were investigated. At these sites, plants of *T. capillaris* and standardized cultures of *L. multiflorum* were exposed to ambient air. Foliar concentrations of PAHs (16 priority pollutants according to US-EPA) and of the trace elements Br, Co, Cu, Fe, Mn, Ni, Pb and Zn were determined. A high level of vehicular traffic was associated with the largest concentrations of PM_{10} in ambient air and with the highest contents of PAHs and heavy metals in the bioindicator plants. The results showed a similar pattern between *T. capillaris* and the standardized biomonitor *L. multiflorum*. Therefore, these results allow us to propose *T. capillaris* as a suitable bioindicator to assess the distribution of pollution impacts caused by PAHs and trace elements in different subtropical and tropical regions.

1. Introduction

Air pollution is one of the most important environmental problems in densely populated and industrialized areas. Airborne particulate matter (PM) in urban air has been at the center of recent concerns, mainly due to its adverse health effects on the urban population. In fact, there are numerous references that indicate a direct relationship between exposure to atmospheric particulate matter and health problems (Pope et al., 2002; WHO, 2003). Among the characteristics of particulate matter that relate to its toxicity may be mentioned the contents of trace metals (Dye et al., 1999), black carbon (Laden et al., 2000), endotoxins (Soukup and Becker, 2001), and the presence of polycyclic aromatic hydrocarbons (PAHs) and other organic compounds (Monn and Becker, 1999). Of these pollutants, trace metals and PAHs play a major role in the toxicity and ecotoxicity of dust particles, thereby producing adverse health effects (Götschi et al., 2005). In addition, ionisable or bioavailable metals in airborne PM have been associated with enhanced airway hyperresponsiveness, altered immune resistance and pulmonary inflammation (Costa and Dreher, 1997). A great number of PAHs have been shown to produce mutagenic or carcinogenic effects and many others may act as co-carcinogens or tumor promoters (Luch, 2005). Motor vehicles have been demonstrated to be a major contributor of particle-bound trace metals (Manalis et al., 2005; Valavanidis et al., 2006) and PAHs (Howsam and Jones, 1998; Van Metre and Mahler, 2003) in urban areas.

The EU directive 2004/107/EC has identified benzo(a)pyrene as a marker of the carcinogenic risk of PAHs. This legislation also establishes the use of bioindicators for assessing the impact of these pollutants on the ecosystem (EU, 2004). However, due to atmospheric concentrations of trace metals and PAHs being generally too low to cause easily detectable effects by visible injury symptoms, accumulative bioindicator plants have been used for biomonitoring of particle-bound air pollutants (Klumpp and Ro-Poulsen, in press). PAHs occurring in the atmosphere in the vapor and/or particulate phase are deposited to the vegetation via dry and wet deposition. Whereas substances in the vapor phase can be absorbed by stomatal uptake and/or diffusion through wax layers and membranes, particle-bound PAH compounds accumulate in the lipophilic cuticular wax layer on the leaf surface (Holoubek et al., 2000; Lehndorff and Schwark, 2004).

Among the plant species previously utilized for biomonitoring of PAHs are mosses (Orlinski, 2002), herbaceous species (Franzaring and van der Eerden, 2000; Bakker et al., 2001), aquatic macrophytes (Söderström and Bergqvist, 2003), and tree species (Lehndorff and Schwark, 2004). The standardized culture of the

* Corresponding author. Tel.: +49 711 45923043; fax: +49 711 45924347.
E-mail address: andreas.klumpp@uni-hohenheim.de (A. Klumpp).

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grass species *Lolium multiflorum* ssp. *italicum* cv. Lema is one of the bioindicators most used to detect accumulation of trace elements, sulfur, fluorides and organic pollutants in urban centers in Europe as it provides reliable information on the environmental change caused by human activities over a short time (Klumpp et al., 2009). Epiphytic species of the genus *Tillandsia* which are very common in the Southern Hemisphere, have also frequently utilized for the biomonitoring of airborne trace metals (Schrimpff, 1984; Pignata et al., 2002; Wannaz and Pignata, 2006; Figueiredo et al., 2007; Bermudez et al., 2009). These plants possess peculiar specialized epidermal structures, so-called trichomes, which absorb aerosols and particles together with water directly from the air, whereas their roots retain only the function of anchoring the epiphyte to the substrate (Brighigna et al., 1997, 2002). Besides, a recent study on the ultrastructure of *Tillandsia* trichomes described apoptotic mechanisms at maturity acting as a passive pump and thus achieving an important function in the absorption mechanism (Papini et al., 2010). In addition, *T. usneoides* has been applied as a PAH bioindicator in Brazil (De Souza Pereira et al., 2007), and *T. caput-medusae* as well as *T. bulbosa* have been employed as PAH bioindicators in Italy (Brighigna et al., 2002).

The purpose of this research was to analyze the relationship between the accumulation of PAHs and trace elements in the bioindicator plant *T. capillaris* with different levels of vehicular traffic in the city of Stuttgart (Germany), and also to compare the accumulation of trace elements in *T. capillaris* with that of the standardized biomonitor *L. multiflorum*.

2. Materials and methods

2.1. Biological material, collection procedures and cultivation of standardized grass cultures

Plants of *Tillandsia capillaris* Ruiz and Pav. form *capillaris* were collected from tree trunks in San Isidro, Cordoba Province, Argentina (31°48′55″S, 64°24′04.7″W). This area is characterized by low emissions of air pollutants.

Italian ryegrass [*L. multiflorum* (LAM) ssp. *italicum* cv. Lema] cultures were grown in a greenhouse, strictly adhering to the standard protocol of VDI (2003). 0.6 g of grass seeds were sown in plastic pots (A = 15.4 cm^2) filled with non-fertilized cultivation substrate (ED73 Type 0), 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.

Plant watering in the field was done automatically using glass fiber wicks (ø 5 mm) and a water reservoir of deionized water of 5 L. On the day set for plant exposure, the grass cultures were cut back to 4 cm and fertilized once more. 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. Study area and choice of sites

The study was performed in Stuttgart (S-Germany) and the neighboring town of Filderstadt. Although the city of Stuttgart only has a population size of about 600 000 inhabitants, when counting the neighboring cities the Greater Stuttgart region has a population of c. 2.8 million inhabitants and is one of the most densely populated and industrialized regions in Germany. The city center is located in the wide Neckar basin at about 200 m asl, with the municipal area extending to the surrounding hills and reaching an elevation of about 500 m asl. The climate is mild, with an average annual temperature of about 8.6–10.6 °C and an average annual rainfall of 670–720 mm, depending on the elevation.

The bioindicator network covered 10 sites with different levels of vehicular traffic as well as areas with few pollutant emissions. In addition, these areas were characterized by the type of environment (urban/traffic, urban/background, traffic, suburban/background and rural) (Fig. 1). Nine out of the 10 sites corresponded to the automated monitoring stations run by the Environmental Protection Agency of the State of Baden-Württemberg (LUBW).

Fig. 1. Map showing the location of the monitoring stations in the Greater Stuttgart region, Germany (original source: Google Earth, 2009). These stations were characterized by the type of environment (◎) urban/traffic, (△) urban/background, (⊙) traffic, (⊡) suburban/background and (○) rural.

These stations were classified as: (a) permanent stations with continuous measurements of NO_2, O_3, PM_{10}, precipitation, temperature, wind speed and direction, and (b) temporary stations, located on the streets, with continuous measurements of PM_{10} and NO_2.

2.3. Exposure methods

Bags containing 300 g of *T. capillaris* and pre-cultivated cultures of *L. multiflorum* were transplanted to 10 and eight sites, respectively, with different levels of vehicular traffic, from 6 June to 29 August, 2007. Exposure of *T. capillaris* and *L. multiflorum* was performed according to Wannaz and Pignata (2006) and VDI (2003), respectively.

During the 12 week experimental period, grass cultures were exposed at these sites for three consecutive periods of 4 weeks each. After each exposure period, the cultures were sampled and replaced by new ones. However, in the case of the bags of *T. capillaris*, these were transplanted to each monitoring station once during a longer continuous experimental period of 12 weeks.

2.4. Characterization of the monitoring stations

Each monitoring site was graded with respect to a set of environmental variables associated to the traffic level. The rankings were defined as follows:

- High: with trucks, urban transportation trains and buses; sites 2, 5 and 8.
- Moderate: private cars, urban transportation trains and buses; sites 3, 4 and 7.
- Low: scarce vehicular traffic of mainly private cars; sites 1, 9 and 10.

2.5. Sample preparation and chemical analysis

2.5.1. T. capillaris

After the exposure period, plants of *T. capillaris* were collected and stored at −80 °C until further analysis. Sub-samples of c. 100 g FW were taken and dried at 60 °C to determine the DW/FW ratio.

2.5.2. L. multiflorum

At each site, the plant biomass of the grass cultures per site was harvested. A PVC ring, 4 cm high, was put over the grass and used as a spacer. The grass was carefully cut, transferred into labeled paper bags and kept in a cooling box. After transfer to the lab, the fresh weight of each sample was determined. The grass samples were then dried at 60 °C for 48 h and their dry weights were determined.

2.5.3. Extraction, cleanup and analysis of PAHs

Determinations of the 16 PAH priority pollutants defined by US-EPA were performed by the Agricultural Research Institute LTZ Augustenberg, Germany, according to the standard procedure of the Association of German Agricultural Investigation and Research Centers (VDLUFA) based on Speer et al. (1990) and described in detail by Trenkle and Janßen (2003). The extraction procedure was performed by placing the homogenized material with an aliquot of internal standard solution and mixed with water, acetone and petroleum ether (1:2:1 v/v) using 80 g of NaCl. The extracts were homogenized and then the supernatant organic phase was dried with sodium sulfate, filtered and concentrated to 0.5 mL in a rotary evaporator with the thermostatic bath at max. 40 °C.

The samples were further cleaned up using gel permeation chromatography (GPC). To this end, the remaining solvents were evaporated with a gentle flow of nitrogen. Then the residue was eluted with 10 mL of cyclohexane/ethylacetate (1:1, v/v), and 5 mL of the eluate applied to the GPC. The eluate was collected and again concentrated to 0.5 mL in a rotary evaporator at max. 40 °C. After cautious expulsion of the remaining solvent under a weak nitrogen flow the eluate was then further purified through adsorption chromatography (AC) with silica gel using petrolether and dichloromethane (4:1 v/v). After a further concentration and drying process as above, the eluate was mixed with propan-2-ol and finally purified on a Sephadex® LH-20 column. The eluate was collected, concentrated and dried under a weak nitrogen flow. The residue was dissolved in 0.5 mL cyclohexane.

The qualitative and quantitative determinations were carried out by means of a gas chromatograph (Hewlett–Packard HP 6890) equipped with a cooled injector system (Gerstel, CIS 4) and coupled to a mass spectrometer (HP 5973) operating with acquisition data (Software G1701BA, Version B.00.00). GC separations were performed on an HP-5 MS® (30 m × 0.25 mm i.d., 0.25 μm) fused-silica capillary column. The injector mode was splitless (1 min), with a total flow of 54.3 mL min^{-1}. The injector temperature was maintained at 290 °C and the GC temperature program was as follows: 90 °C for 4 min; rising to 100 °C at a rate of 10 °C min^{-1}; rising from 100 °C to 290 °C at a rate of 3 °C min^{-1}; maintained at 290 °C for 22 min. The carrier gas was ultra-pure helium at constant flow of 1 mL min^{-1}. The mass spectrometer was operated in EI mode, the detection was made in selected ion monitoring (SIM) mode. The selected ions and dwell times are reported in Table 1.

Calibration was done by injecting blanks, a standard mixture of individual PAH compounds and deuterated acenaphthene, benzo(a)pyrene, and phenanthrene as internal standards. The identification of individual components of samples and standard mixture was carried out by comparing ion mass and dwell times (Table 1). To verify the reliability of the analytical procedure, ring tests with various reference plant materials were performed in advance. The detection limit of the analytical method was 0.1 μg kg^{-1} FW plant material for each PAH compound.

2.5.4. Elemental analysis of plant samples

For the determination of Br, Co, Cu, Fe, Mn, Ni, Pb and Zn concentrations in leaves of *T. capillaris* and *L. multiflorum,* the dried

Table 1
List of PAH components, internal deuterated standards (underlined), quantification and confirmation ions and dwell times.

PAH component	Quantification ion	Confirmation ions	Dwell time
Naphthalene	128	129, 126	15
Acenaphthylene	152	153, 151	10
D_{10}- Acenaphthene	164	162, 160	10
Acenaphthene	154	155, 150	10
Fluorene	166	165, 163	15
D_{10}- Phenanthrene	188	189, 187	10
Phenanthrene	178	179, 176	10
Anthracene	178	179, 176	10
Fluoranthene	202	203, 200	15
Pyrene	202	203, 200	15
Benz(*a*)anthracene	228	229, 226	25
Chrysene	228	229, 226	25
Triphenylene	228	229, 226	25
Benzo(*b*)fluoranthene	252	253, 250	25
Benzo(*k*)fluoranthene	252	253, 250	25
D_{12}- Benzo(*a*)pyrene	264	265, 260	10
Benzo(*a*)pyrene	252	253, 250	10
Indeno(1.2.3-*cd*)pyrene	276	279, 274	10
Dibenz(*a.h*)anthracene	278	279, 274	10
Benzo(*ghi*)perylene	276	277, 274	15

plant material was ground and reduced to ashes at 500 °C for 4 h. These ashes were digested with HCl (18%):HNO_3 (3:1), before 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, and 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. For the excitation, a polychromatic beam was used of approximately 0.3 mm wide and 2 mm high. For the X-ray detection, a Si(Li) detector was used with an energy resolution of 165 eV at 5.9 keV.

As a quality control, blanks and samples of the standard reference material "Hay IAEA-V-10" were prepared in the same way and were run after five determinations to calibrate the instrument. The results were found to be within ±2% of the certified value. The coefficient of variation of replicate analysis was calculated for different determinations. Variations were found to be <10%. Standard solutions with known concentrations of different elements and Ge as an internal standard were prepared for the calibration of the system.

2.6. Statistical analysis

A one-way analysis of variance (ANOVA) for PM_{10}, PAHs and trace elements was carried out considering the levels of vehicular traffic (high, moderate or low). A pairwise comparison of means by the Tukey test was performed whenever the ANOVA indicated significant effects ($p < 0.05$). The ANOVA assumptions were previously verified graphically (residual vs. fitted values, box plots, and steam leaf plots).

A principal component analysis (PCA) was performed using the levels for the vehicular traffic classification criteria in order to assess the relationship between them and the accumulation of elements of each species studied.

3. Results and discussion

3.1. Air pollution and climatic conditions during the study period

The hourly limit value of 200 μg NO_2 m^{-3} for the protection of human health (EU, 1999), which should not be exceeded more than

Table 2
Mean values of the concentrations ± standard deviation (SD) and results of the analysis of variance (ANOVA) of PM_{10} (μg m^{-3}) measured in the automated monitoring stations and of PAHs (μg kg^{-1} DW) determined in *T. capillaris*, between the levels of vehicular traffic (high, moderate, low) in the city of Stuttgart, Germany.

Variables	Vehicular traffic			
	High	Moderate	Low	ANOVA
PM_{10}	24.7 ± 7.47 a	20.6 ± 0.44 ab	15.3 ± 4.89 b	*
Naphthalene	1.86 ± 0.88	2.52 ± 2.15	0.47 ± 0.28	ns
Acenaphthylene	11.9 ± 2.15 a	5.96 ± 2.18 b	3.93 ± 0.79 b	**
Acenaphthene	67.9 ± 25.4 a	29.9 ± 15.0 ab	18.6 ± 10.1 b	*
Fluorene	50.2 ± 17.4 a	20.9 ± 6.15 b	8.48 ± 4.30 b	**
Phenanthrene	595 ± 293 a	263 ± 122 ab	98.6 ± 29.5 b	*
Anthracene	21.8 ± 5.80 a	10.2 ± 4.43 b	6.25 ± 1.57 b	**
Fluoranthene	614 ± 149 a	335 ± 258 ab	128 ± 43.5 b	*
Pyrene	311 ± 47.0 a	154 ± 107 ab	58.2 ± 17.9 b	*
Benz(*a*)anthracene	29.8 ± 11.2 a	12.5 ± 5.10 b	6.59 ± 0.80 b	*
Triphenylene + Chrysene	199 ± 81.2 a	91.2 ± 34.1 ab	52.4 ± 11.4 b	*
Benzo(*b*)fluoranthene	50.8 ± 15.6 a	22.5 ± 6.36 b	16.4 ± 1.63 b	**
Benzo(*k*)fluoranthene	16.6 ± 2.14 a	9.33 ± 2.82 b	9.03 ± 3.29 b	*
Benzo(*a*)pyrene	22.4 ± 8.58 a	10.8 ± 4.36 ab	7.38 ± 2.37 b	*
Indeno(1.2.3-*cd*)pyrene	12.7 ± 6.27	6.49 ± 4.63	nd	–
Dibenz(*a.h*)anthracene	10.3 ± 1.08	nd	nd	–
Benzo(*ghi*)perylene	65.2 ± 8.11 a	36.3 ± 21.0 ab	8.36 ± 3.69 b	**

ANOVA between levels of vehicular traffic. Significantly different means at $p < 0.05$ within one row are indicated by different letters. nd. Not detected, ns. Not significant.
* $p < 0.05$,
** $p < 0.01$.

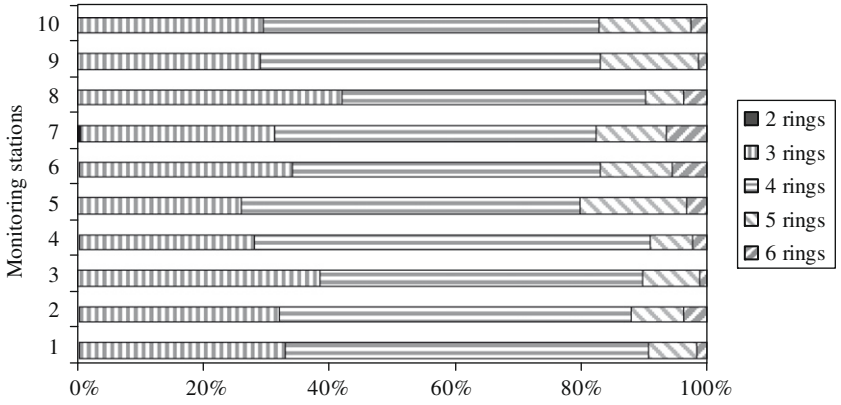

Fig. 2. Percentage fractions of 2, 3, 4, 5 and six rings of PAHs in *T. capillaris*.

18 times per year, was surpassed at stations 5 (Neckartor I) and 8 (Hohenheimer Str.) 411 times and 261 times, respectively, during our study period. The O_3 exposure index for the protection of vegetation (AOT40 of 18 000 μg m^{-3} h) was not exceeded at any of the sites.

The mean temperature during the whole exposure period ranged from 17.7 (site 10) to 19.1 (site 3), with a mean minimum temperature of 11 °C and a mean maximum temperature of 26–28 °C. The accumulated precipitation varied between 188 mm (site 3) and 244 mm (site 10).

The atmospheric particulate matter concentrations (PM_{10}) in each of the monitoring stations did not exceed the limits for the annual mean of 40 μg PM_{10} m^{-3} (EU, 1999).

The daily limit of 50 μg PM_{10} m^{-3}, which, for the protection of human health, should not be exceeded more than 35 times per year was surpassed at stations 2 (3 d), 5 (5 d) and 8 (1 d) during this study.

Table 3
Mean values of the concentrations ± standard deviation (SD) and results of the analysis of variance (ANOVA) of metals and Br determined in *T. capillaris* between the levels of vehicular traffic (high, moderate, low) in the city of Stuttgart, Germany.

Elements	Vehicular traffic			ANOVA
	High	Moderate	Low	
Mn (μg g^{-1} DW)	236 ± 22.2 a	254 ± 28.8 a	159 ± 21.9 b	***
Fe (mg g^{-1} DW)	7.21 ± 0.71 a	6.08 ± 0.98 ab	5.12 ± 0.59 b	**
Co (μg g^{-1} DW)	2.06 ± 0.59	3.27 ± 1.62	2.16 ± 0.65	ns
Ni (μg g^{-1} DW)	17.7 ± 10.5	13.7 ± 10.3	6.14 ± 4.27	ns
Cu (μg g^{-1} DW)	46.8 ± 22.2 a	29.5 ± 14.2 ab	11.5 ± 3.80 b	**
Zn (μg g^{-1} DW)	178 ± 60.3 a	102 ± 32.6 b	47.3 ± 10.8 b	***
Br (μg g^{-1} DW)	11.2 ± 1.89 ab	14.6 ± 4.14 a	9.27 ± 3.33 b	*
Pb (μg g^{-1} DW)	4.86 ± 1.32 b	6.72 ± 0.82 a	4.61 ± 0.46 b	**

ns. Not significant,
ANOVA between levels of vehicular traffic.
* $p < 0.05$,
** $p < 0.01$,
*** $p < 0.001$.

3.2. Polycyclic aromatic hydrocarbons

3.2.1. Total and individual PAH concentrations in T. capillaris

The total PAH contents in the samples ranged from 330 (site 9) to 2650 (site 8) μg kg^{-1} DW, which is in agreement with results of other studies (Brighigna et al., 2002; De Souza Pereira et al., 2007).

The lowest value referred to site 9, Uni – Heidfeldhof, the least contaminated site, which was taken as a reference site. The highest concentration was measured at site 8, Hohenheimer Str., an urban area with a high traffic load. High PAH contents were also measured at sites 5, 2 and 4 (Neckartor I, Feuerbach and Bad Cannstatt II), respectively, located in an urban area with high or moderate vehicular traffic. Related to this, there are numerous references in the literature concerning motor vehicle emissions as the main source of PAHs in urban areas (Lim et al., 1999; Lehndorff and Schwark, 2004). There were no major stationary emission sources of PAHs such as power, waste incineration and industrial plants in the vicinity of the exposure sites, although such sources cannot be completely excluded in densely built urban areas. Domestic heating as another important PAH source in cities can be excluded as the present study was conducted during summertime. The predominant PAH compounds at all monitoring sites were phenanthrene, and, to a lesser extent, fluoranthene, pyrene and triphenylene + chrysene (Table 2). This is in agreement with previous research which established a relationship between these hydrocarbons and vehicular emissions (Lehndorff and Schwark, 2004; Brachtl et al., 2009).

3.2.2. Aromatic rings in the PAH compounds accumulated by T. capillaris

The compounds were grouped into classes according to the number of aromatic rings present in their structures, with the purpose of establishing a relationship between the volatility of the compounds and the monitoring sites. For all these sites, 3- and 4-ring compounds (higher or medium volatilility) ranged from

Table 4
Mean values of the concentrations ± standard deviation (SD) and results of the analysis of variance (ANOVA) of metals and Br measured in *L. multiflorum* between the levels of vehicular traffic (high, moderate, low) for the three exposure periods in the city of Stuttgart, Germany.

Elements	Exposure period	Vehicular traffic			ANOVA
		High	Moderate	Low	
Mn	1	184 ± 39.7 a	84.6 ± 22.7 b	83.2 ± 11.5 b	***
(μg g^{-1} DW)	2	128 ± 2.72 b	205 ± 75.0 a	80.1 ± 11.7 b	**
	3	203 ± 54.2	216 ± 27.5	209 ± 160	ns
Fe	1	1.82 ± 0.02 a	0.92 ± 0.65 b	0.57 ± 0.74 b	***
(mg g^{-1} DW)	2	1.55 ± 0.12 ab	3.16 ± 1.74 a	0.71 ± 0.10 b	**
	3	3.15 ± 0.13	1.34 ± 0.70	3.38 ± 4.16	ns
Co	1	2.85 ± 1.74 a	0.73 ± 0.38 b	0.69 ± 0.23 b	**
(μg g^{-1} DW)	2	1.59 ± 1.12 ab	2.68 ± 1.00 a	0.51 ± 0.31 b	**
	3	2.40 ± 0.94	1.75 ± 0.16	4.44 ± 3.49	ns
Ni	1	11.1 ± 9.01	8.22 ± 7.02	3.24 ± 1.12	ns
(μg g^{-1} DW)	2	8.61 ± 1.74 b	14.9 ± 5.72 a	3.77 ± 1.62 b	**
	3	6.69 ± 4.43	5.41 ± 1.47	11.9 ± 7.30	ns
Cu	1	35.5 ± 3.5 a	17.0 ± 11.9 b	8.03 ± 1.15 b	***
(μg g^{-1} DW)	2	31.9 ± 9.87 a	28.1 ± 12.0 a	7.18 ± 4.44 b	**
	3	44.6 ± 7.05 a	34.5 ± 20.8 ab	14.1 ± 4.62 b	**
Zn	1	94.1 ± 2.42 a	53.33 ± 20.35 b	29.9 ± 6.89 c	***
(μg g^{-1} DW)	2	73.7 ± 7.84 a	64.8 ± 20.5 a	26.4 ± 9.38 b	***
	3	111 ± 3.79 a	71.6 ± 19.8 b	46.3 ± 15.9 b	***
Br	1	16.3 ± 5.19 a	7.33 ± 0.79 b	5.44 ± 0.79 b	***
(μg g^{-1} DW)	2	11.4 ± 3.00 a	10.8 ± 1.20 a	6.69 ± 2.17 b	*
	3	11.0 ± 3.72	15.7 ± 1.80	10.4 ± 3.37	ns
Pb	1	4.94 ± 1.67 a	2.33 ± 0.03 b	1.83 ± 0.54 b	**
(μg g^{-1} DW)	2	4.11 ± 1.60 a	2.79 ± 0.08 ab	1.44 ± 0.32 b	**
	3	4.31 ± 1.66	4.12 ± 0.13	4.01 ± 1.93	ns

ns. Not significant,
ANOVA between levels of vehicular traffic.
* $p < 0.05$,
** $p < 0.01$,
*** $p < 0.001$.

80% to 90% of the total, while 5- and 6-ring compounds (lower volatilility), which have a high carcinogenic potency such as benzo(*b*)-fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenzo(*a,h*)-anthracene and indeno[1,2,3-*cd*]pyrene (US-EPA, 1993), were more frequent in the plants of those sites with high vehicular traffic (Fig. 2).

3.2.3. Correlation between PM_{10} and PAH compounds accumulated in *T. capillaris*

The correlations between the PM_{10} values and the contents of the 16 EPA-PAH were determined in *T. capillaris* leaves. Most results showed a significant ($p > 0.05$) positive correlation for: acenaphthylene (0.84), anthracene (0.72), pyrene (0.70), benz(*a*)anthracene (0.75), triphenylene + chrysene (0.70), benzo(*b*)fluoranthene (0.75), benzo(*k*)fluoranthene (0.69), benzo(*a*)pyrene (0.78), and benzo(*ghi*)perylene (0.82). The high Pearson correlation coefficient between the majority of these hydrocarbons and PM_{10} indicates that *T. capillaris* is efficiently accumulating these pollution compounds from particulate matter and may therefore be considered a suitable bioindicator for particle-bound PAHs.

Table 5
Eigenvectors obtained in principal component analysis of the variables measured in *T. capillaris* and *L. multiflorum*.

Variables	*T. capillaris*		*L. multiflorum*	
	Component 1	Component 2	Component 1	Component 2
Mn	0.43	0.14	0.35	−0.35
Fe	0.38	−0.29	0.38	0.16
Co	0.17	0.55	0.25	0.68
Ni	0.42	−0.17	0.30	−0.56
Cu	0.40	−0.26	0.38	−0.06
Zn	0.37	−0.31	0.38	0.06
Br	0.33	0.40	0.38	−0.07
Pb	0.24	0.50	0.37	0.24
Eigenvalues	5.14	2.86	6.76	1.24

3.2.4. PM_{10}, PAHs and vehicular traffic

Table 2 shows mean values, standard deviation and ANOVA results of PM_{10} concentrations measured in the automated monitoring stations and 16 PAHs according to the US-EPA measured in *T. capillaris* for different levels of vehicular traffic. For particulate matter and most PAHs, the highest values corresponded to high or moderate vehicular traffic. The results of other studies also showed that traffic was the major determinant for concentrations of particulate matter and PAHs in urban air (Valavanidis et al., 2006).

3.3. Trace elements in *L. multiflorum* and *T. capillaris*

Tables 3 and 4 display mean values, standard deviation and ANOVA results of heavy metals and Br measured in leaves of

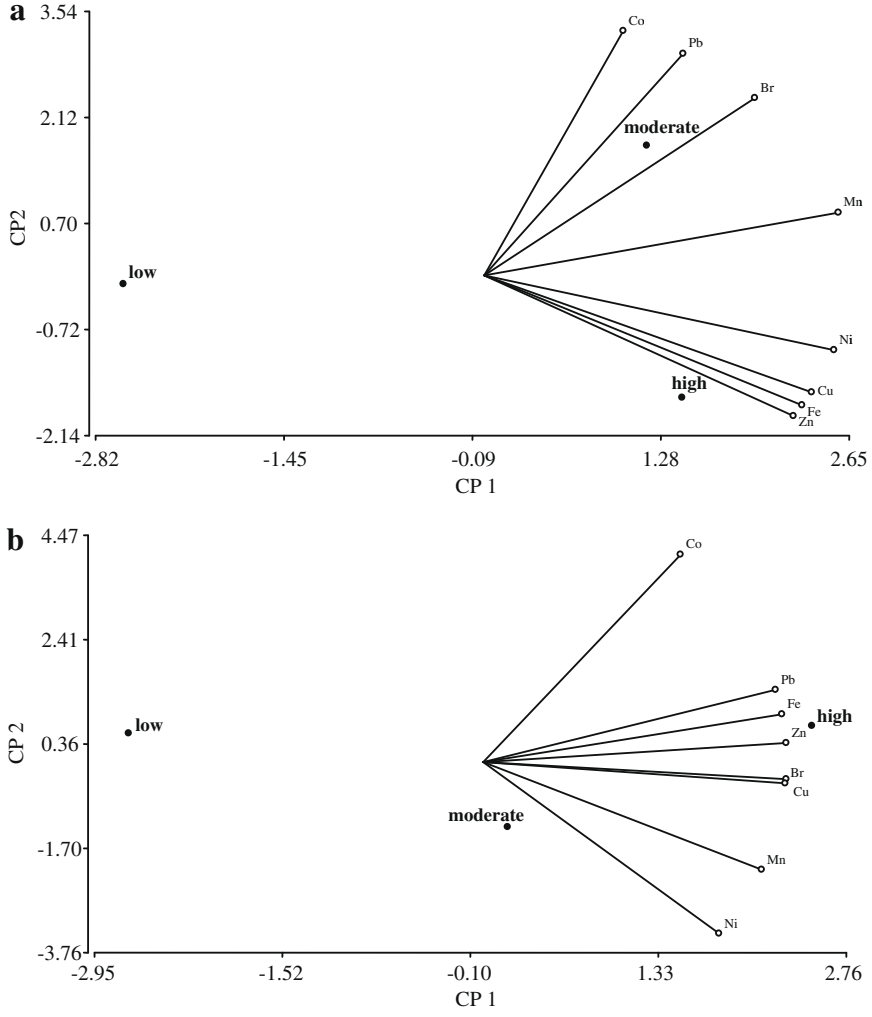

Fig. 3. Biplots based on the two principal components of the principal component analysis for heavy metal concentrations and accumulated Br in *T. capillaris* (Fig. 3a) and *L. multiflorum* (Fig. 3b), using the three levels of vehicular traffic (high, moderate, low) as the classification criteria in the city of Stuttgart, Germany.

T. capillaris and *L. multiflorum*. These are grouped into sites with different levels of vehicular traffic. In general, the bioindicator plants showed the strongest accumulation of trace elements at sites with high or moderate vehicular traffic load. In various studies, increased contents of Cu, Mn, Ni, Pb and Zn in plants have clearly shown an association with intense vehicular traffic, probably mainly due to abrasion from brakes and the car body, and also resulting from fuel combustion (Davis and Williams, 1975; Ward, 1990; Riveros-Rosas et al., 1997; Harrison et al., 2003; Zechmeister et al., 2006). The presence of Br may be attributed to long-range transport and fossil fuel use (Lammel et al., 2002). Moreover, the occurrence of high levels of Fe and Co may be associated with resuspended road dust (Zechmeister et al., 2006).

A principal component analysis (PCA) was performed in order to evaluate the element accumulation in the bioindicators. This analysis was undertaken using the levels of vehicular traffic as the classification criteria. Eigenvalues corresponding to the first two components are displayed in Table 5, with the results being presented in biplots (Fig. 3a and b) for *T. capillaris* and *L. multiflorum*, respectively.

The results obtained for *T. capillaris* showed a positive association between the contents of Zn, Fe, Cu, Ni and high vehicular traffic, while the contents of Br, Pb and Co were associated with moderate vehicular traffic.

The elements Zn, Fe, Pb, Br and Cu accumulated by the bioindicator *L. multiflorum* showed a positive association with high traffic. To a lesser extent, the elements Ni and Mn also showed a direct association with moderate vehicular traffic.

The PCA clearly confirmed the association of trace element accumulation in both bioindicator species with emissions from car traffic and thus corroborated the conclusions based on the ANOVA. PCA patterns for both species investigated were similar, but not fully identical. This may partly be due to the different exposure duration (12 weeks vs. 3×4 weeks) and hence integration time of the two bioindication methods we used. Additionally, considering morphological and anatomical differences between both species (e.g., presence of trichomes in *T. capillaris*), it can be assumed that they also differ in element absorption and uptake mechanisms as well as in sensitivity to processes causing removal of deposited material (e.g., wind abrasion, wash-off). Root uptake of trace elements deposited to the soil surface of grass cultures, by contrast, can be mostly neglected according to previous studies (VDI, 2003).

4. Conclusions

The results obtained in this study in relation to the three levels of vehicular traffic in the city of Stuttgart showed that the highest concentration of PM_{10} (instrumental measurement system), PAHs and trace elements (accumulated by bioindicators) were related to urban areas with high vehicular traffic. The suitability of *T. capillaris* (Ruiz and Pav.) form *capillaris* as an accumulation bioindicator for trace elements was also confirmed in this study in a region where the transplanted species was absent, which showed a similar response pattern to the standardized bioindicator *L. multiflorum* (LAM) cv. Lema. Furthermore, *T. capillaris* proved to be a suitable and sensitive biomonitor for the accumulation of polycyclic aromatic hydrocarbons. It can therefore be used to establish distribution patterns with respect to impacts and possible emission sources of these compounds in tropical and subtropical regions of the world.

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