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Evaluation of the suitability of *Tillandsia usneoides* (L.) L. as biomonitor of airborne elements in an urban area of Italy, Mediterranean basin

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ABSTRACT

The suitability of a rootless Bromeliad species (*Tillandsia usneoides*) as biomonitor of airborne trace elements in urban areas of the Mediterranean basin was evaluated. The study was performed at five sites of the city of Pisa (Tuscany, Central Italy) differing for land use, anthropogenic activities and/or proximity to emission sources. The elements investigated were Al, As, B, Ba, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sb, Sr, V and Zn. Unwashed and washed samples, collected after 36 days of exposure (May–June 2011), were analyzed by ICP–MS. Results showed significant differences among sampling sites for several elements. Concentrations of Al, Ba, Bi, Cd, Co, Cu, Pb, Sb and Zn were the highest in urban/traffic and/or suburban/traffic areas. Some of these elements e.g. Ba, Cu, Sb and Zn are commonly considered as traffic–related elements. In the industrial site, the main elements found were Mg, Sr and Zn. Iron, Mn, Na and V concentrations were much higher in rural/remote areas. Enrichment factors highlighted that *T. usneoides* showed: high resistance/tolerance to heavy metal toxicity, specificity, capability to well–definitely represent a sampling site, quantitative response to pollutant exposure. The results indicated that *T. usneoides* reflects the intrinsic characteristics of each sampling area and allows tracing back differences related to the various emission sources by factor analysis.

Keywords: *Biological monitoring, enrichment factor, factor analysis, Spanish moss, traffic–related elements*

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1. Introduction

Interest in air pollution and its consequences for human health has reached an extraordinary extent in recent years (Kampa and Castanas, 2008). The different composition of air contaminants and the fact that living organisms are usually exposed to pollutant mixtures rather than to single substances, have led to problems for identifying and attributing the potential effects of pollution to specific sources. The major source of information on this topic can be obtained by field measurements of emissions or dispersion modeling but these measurements are of minimal use to evaluate biological impact of pollutants and are mostly performed on restricted geographical scale (Wolterbeek, 2002). The use of transplants and cultivated indicators for environmental diagnosis should be regarded as an efficient supplement and a necessary complementary tool to integrate the aforementioned type of investigations, because these plants can be used to describe the presence of harmful emissions in the air in relation to changes in their behavior and the bioavailability of a given contaminant in their tissues.

In the literature, biomonitoring species for airborne contaminants are often selected on the basis of criteria such as: specificity, well–defined representation of a sampling site and accumulation ratios. Lichens (Conti and Cecchetti, 2001; Salo et al., 2012; Chaparro et al., 2013) and mosses (Aceto et al., 2003; Fabian et al., 2011) have been widely used as biondicators to map atmospheric trace elements deposition because, in the presence of anomalous concentrations of chemical species, they are subjected to morphological and/or physiological changes due to stress conditions. Recently, other researchers proposed the use of vascular plants as bioindicators and bioaccumulators of trace elements, particularly in urban and industrial areas, where lichens and mosses are often missing. Leaves of various species have been studied for this purpose: an evergreen ornamental shrub species was used as a passive sampler to describe the spatial distribution of selected elements in three coastal cities of Central Italy (Lorenzini et al., 2006) and in the area of Messina (Sicily, Italy) (Matarese Palmieri et al., 2005). Leaves of the evergreen species were used as indicators of airborne trace element in anthropogenic and remote sites of Tuscany (Central Italy) (Francini et al., 2010a) and Campania (Southern Italy) (Maisto et al., 2004). The foliage of common deciduous trees from the urban area of Belgrade, Serbia (Anicic et al., 2011) was used for long–term monitoring of trace metal concentrations. Nali et al. (2009) used lettuce plants to map the concentration of selected elements at specific sites in a small municipality in Tuscany. *Lolium multiflorum* was chosen as an active bioindicator species of airborne heavy metals in 11 European cities (Klumpp et al., 2009) and in Tuscany (Francini et al., 2010b). The surfaces of deciduous tree leaves from 36 sites around an elevated stack point source, Oxfordshire, UK (Mitchell et al., 2010) were used for biomagnetic monitoring of fine–grained pollutant particles with aerodynamic diameters below 10 µm in diameter (PM_{10}). However, some authors believe that higher plants do not have the due adaptation ability and are not enough geographically widespread (Polechonska et al., 2013).

Tillandsia usneoides (L.) L. (Spanish moss), an epiphytic member of *Bromeliaceae*, is a well–known bioaccumulator of trace elements, but few studies [all of these were run in America, e.g.

Martinez–Carrillo et al. (2010) and Rodriguez et al. (2011)] report the use of this species as atmospheric biomonitor. To the best of our knowledge, even less papers are concerning its use in Europe (Brighigna et al., 2002).

Thus, the aim of this work was to evaluate the bioindicator/biomonitor (Markert, 2008) suitability of Spanish moss in urban areas of the Mediterranean basin, verifying: high resistance/tolerance to heavy metal toxicity, specificity (i.e. the accumulation occurs from the atmosphere), capability to well–definitely represent a sampling site, quantitative response to pollutant exposure. The results of an active biomonitoring survey of air quality carried out in the urban area of Pisa (Tuscany, Central Italy) are reported herein, in order to verify, whether this species can actually be used as a bioindicator/biomonitor also in the Mediterranean area. Particular emphasis was given to trace elements because of their toxic effects on humans (Fraga, 2005).

2. Materials and Methods

2.1. Study area

The city of Pisa (88 000 inhabitants) is located in Tuscany, Central Italy, along the River Arno, ca. 10 km from the Tyrrhenian sea at a mean elevation of 4 m a.s.l. The economical structure is mainly based on industrial activities, service sector and tourism: in the NE, land use is typically residential and commercial; this area includes many public buildings, offices and shopping centers where almost all local bus and train lines run through; in the SE there is an industrial area, where several small– and medium–sized enterprises are located and an urban waste incinerator. In this area, the international airport "Galileo Galilei" is also located. The area to the W is mainly agricultural (cereal crops) and industrial (mainly shipyards) and includes the nature Reserve of San Rossore.

2.2. Sampling sites

Five sampling sites were selected after a preliminary study, taking into account the location of the most important emission sources and their heterogeneity, the distribution of mobile sources and the direction of prevailing winds (Figure 1). For each sampling site, the details of the land use, anthropogenic activities and/or proximity to emission sources are described:

(1) Suburban/traffic (ST): the sampling site was in via Francesco de Sanctis, a secondary street located in a densely populated area, surrounded by car–crowded streets, where several bus and train lines run through.
(2) Urban/traffic (UT): via Francesco Lavaggi is a road with intense vehicular traffic, flanked by a nearby highway and railway, located in a residential and densely populated area and in the vicinity of the Pisa International Airport.
(3) Industrial (I): via Galileo Ferraris is a street (with circulation of buses and heavy trucks) located in a suburban area characterized by several SMEs (metal–mechanical factories) and by a solid waste incinerator.
(4) Rural/remote (RR): the plot was in via Vecchia di Marina (San Piero a Grado), a secondary road in a remote area near to the Tyrrhenian sea, characterized by extensive agriculture, in the vicinity of the nature Reserve of San Rossore.
(5) Control site (CS) was a facility with charcoal–filtered air under natural climatic conditions at the field station of the University of Pisa.

2.3. Plant sampling and exposure period

Adult individuals of *T. usneoides* were obtained from a commercial greenhouse, authorized provider of tropical and epiphytic plants in the Central Italy. Each sample for exposure was composed by 10 g of plants (cleaned with distilled water before being transplanted), tied by Teflon strings to a gyrator apparatus of 40 cm tall (8 samples per apparatus) which turned with the wind, so that homogenous contact with air contaminants was guaranteed. Only the younger parts of plants (ca. 20 cm length) were used for this study. In order to avoid sudden frost and abundant rain that characterize the winter and part of the spring of the Tuscany [as confirmed by historical data (1994–2008) for the San Piero a Grado (Pisa) meteorological site], the samples were exposed during the dry season from May 6th 2011. After 5 weeks, a slight reddening of the younger leaves was observed and as this

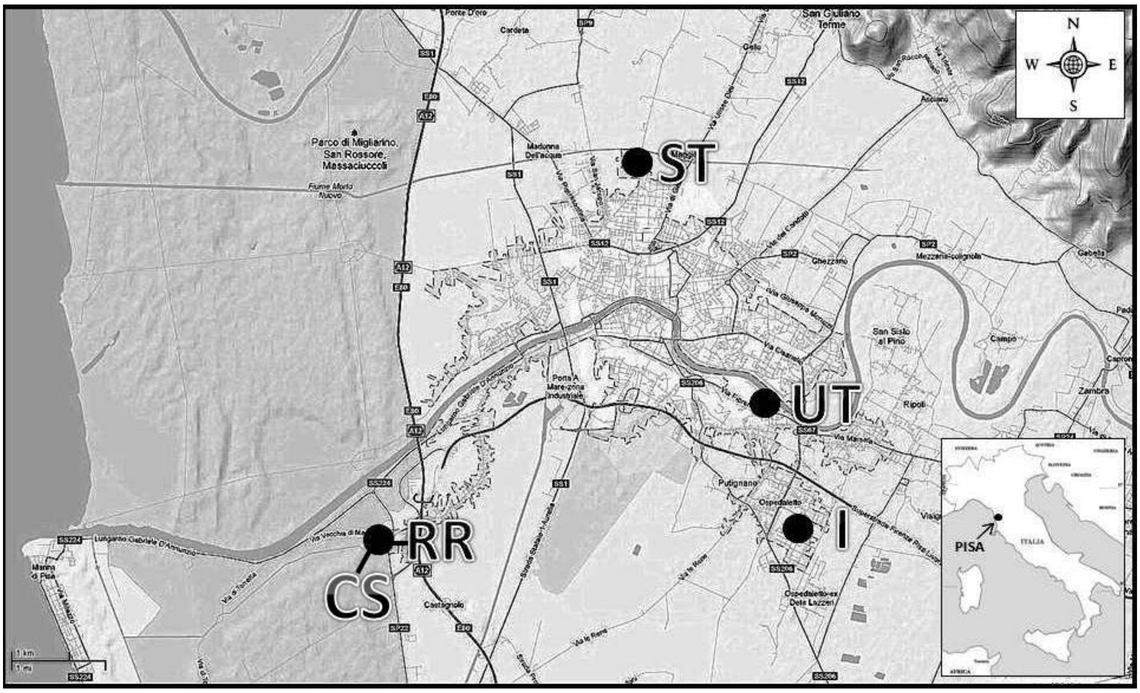

Figure 1. *Map of the Pisa city (Tuscany) with location of sampling sites: control (CS), suburban/traffic (ST), urban/traffic (UT), industrial (I) and rural/remote (RR).*

phenomenon generally precedes the flowering time, the samples were quickly transferred to the laboratory. Material was immediately divided into two aliquots: one was left unwashed and the other was washed with tap water and rinsed 3 times with distilled water to remove surface airborne dust, adsorbed material (see Nali et al., 2009) and the eventual soil particles deposited on the samples via "splash" during same days of precipitation. Concurrent analysis of unwashed and washed samples allows external composition to be distinguished from internal, according to Dasch (1987). Sub–samples were oven dried at 30 °C until constant weight and then crushed to a fine powder and homogenized with a mill.

2.4. Elemental analysis

Total concentrations of Al, As, B, Ba, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sb, Sr, V and Zn in washed and unwashed plant samples were determined. About 10 g of powder were mineralized with a 6:1 (v:v) mixture of ultrapure concentrated HNO_3 and H_2O_2 at 280 °C and a pressure of 0.55 MPa in a microwave digestion system (Milestone Ethos 900). Element concentrations, expressed on a dry weight basis, were determined by Inductively Coupled Plasma–Mass Spectrometry (ICP–MS, Perkin Elmer–Sciex Elan 6100). Analytical quality was checked with the Certified Reference Material GBW07603. Further details are reported in Paoli et al. (2012). Accuracy was within 7% for all elements. All analyses were carried out in triplicate.

2.5. Microscopical observations

At the end of the experiment, plant samples from each site were detached and the micromorphological investigation of foliar structures was done. In particular, ten mid–leaf sections were excised from mature leaves and divided in two aliquots: one was examined under an optical microscope (LEICA DM 4000 B– Leica Microsystems) and the other was immediately fixed in formalin and 70% of acetic acid and maintained in this solution for 48 h. Afterward, five samples of each site, were stored in 70% of acetic acid, according to the technique described by Alves et al. (2008) and examined with stereomicroscope (Wild Heerbrugg Photomakroskop M 400).

2.6. Statistical analysis

Data were evaluated as suggested by Klumpp et al. (2009) which allows the calculation of process–inherent background (B_V) and threshold values (i.e. Effect Detection Limits, EDL). In this procedure, the numerous low values in the study area, which indicate only a low pollution impact, are used as reference values. First, mean values and standard deviation were calculated for the group of sampling sites. Subsequently, single values exceeding a filter threshold (F_j), defined as mean value (xy) plus 1.96 times the standard deviation, were removed from the data collective and the new mean value is calculated. This procedure was repeated until no values exceeded the filter threshold. The remaining values were used as reference values and the arithmetic mean of the reference values was defined as local background value (B_{Vlocal}). EDL was defined as mean background value plus the threefold standard error. The entire procedure has been run according to Francini et al. (2010b). For graphic presentation of the data, box–whisker plots were drawn for each element. Following performance of the Shapiro–Wilk (W) test, differences among stations were evaluated by ANOVA and subsequent LSD post–hoc test. Differences between washed and unwashed samples were checked by the paired–sample *t*–test ($p \leq 0.05$). Relationships between the contents of individual elements were tested using correlation analysis and determination coefficient (R^2) was computed.

To get an indication of the relative contribution of crustal contamination to the bulk element distribution in/on the Spanish moss, enrichment factors (*EF*) were calculated for each element, taking Al as reference element. The average crustal composition reported by Taylor and McLennan (1985) was used in this study. The dimensionless *EF* for any element *X* relative to crustal material is defined by: $EF=(X/Al)_{leaf}/(X/Al)_{crust}$, where $(X/Al)_{leaf}$ is the concentration ratio of *X* to Al in the plant sample, and $(X/Al)_{crust}$ is the average concentration ratio of *X* to Al in the crust. By convention, the average elemental concentration in the crust is used, mostly because particles are subjected to long–range transport phenomena and the characterization of specific areas is not easy (Chester et al., 2000).

The elemental composition of unwashed *T. usneoides* leaves was subjected to Varimax rotated factor analysis (FA), a multivariate statistical treatment frequently used in atmospheric pollution research to obtain information on pollution sources (see Lorenzini et al., 2006).

Analyses were performed by NCSS 2004 Statistical Analysis System Software (NCSS, Kaysville, Utah; see Hintze, 2001).

3. Results and Discussion

3.1. Meteorological characterization

In this study area, the period of May 6 – June 10, 2011 was characterized by a mean temperature of 18 °C that matches rather well with that of historical data (1994–2008) for the San Piero a Grado (Pisa) meteorological site (20 °C). In terms of average rainfalls, this period may be regarded as sufficiently "typical" of the area investigated with a significant reduction (–18 mm) in comparison to the historical series.

3.2. Elemental content

At the end of the experiment, tissues from each site were checked under a stereomicroscope and none of the samples showed morphological alterations. Similarly, abnormalities were not detected in the sectioned samples observed by an optical microscope.

Figure 2 shows an overview of the concentrations of selected elements and their variability across all biomonitoring sites. Washing was effective in reducing the concentrations of many elements in the samples, with 11 (Al, As, Bi, Ca, Cd, Cs, Fe, Mg, Mn, Na and Sr) out of 22 elements showing higher concentrations in unwashed samples, with a removal efficiency ranging from 70–60% (Cd and Bi, respectively) to about 10% (Mg). Similar results have been reported by Aksoy and Ozturk (1997) in leaves of *Nerium oleander* collected in the urban area of Antalya (Turkey). The water–removable proportion of trace elements provides information about both the prevailing element source (natural or anthropogenic) and the leaf absorption amount for each trace element. Results of speciation studies on air particulate deposition of deciduous species suggest that elements from anthropogenic sources are mainly in water–soluble forms (Espinosa et al., 2002). In our study, there are not differences between unwashed and washed samples with regard to Cr, Cu, Pb, Sb and Zn concentrations, although the deposit was removed by water–washing.

A comparison was made between the elemental content of the plants grown in the sampling sites and that of the CS (Table 1). The average concentrations of Cd, Co, Sb, Sr and Zn were significantly ($p \leq 0.05$) higher than those in the controls for unwashed samples (+350, +20, +9, +23 and +72%, respectively); except from Sr, the same situation was observed for washed samples (+50, +27, +30 and +38%, respectively). The average content of elements that are essential micro–nutrients (e.g. Cu, Fe, and Mn) falls in the range of physiological values for plants and agrees with the results of Wannaz et al. (2006). The average concentrations of As, Ba, and Cr were similar to the values reported by Figueiredo et al. (2007) in Stuttgart (Germany), but the concentrations of Al, Co and V were lower than the values measured by these authors. Although the concentrations of Pb and Zn were similar to those found by Rodriguez et al. (2010), the levels of Co, Cu, Fe, Mn, and Ni were lower than those reported by these authors. The mean values found in our study for Cu and Ni were also similar to those found by Martinez–Carrillo et al. (2010) in *T. usneoides*; the concentrations of Cu and Zn were similar to those found by Husk et al. (2004), but these authors found higher mean values of Pb. The lower concentration of Pb found in our study confirmed that the ambient levels of this element have dramatically decreased owing to the ban of leaded gasoline (in Italy complete legal ban started from 1 January 2002) and a generally better regulation of industrial emissions.

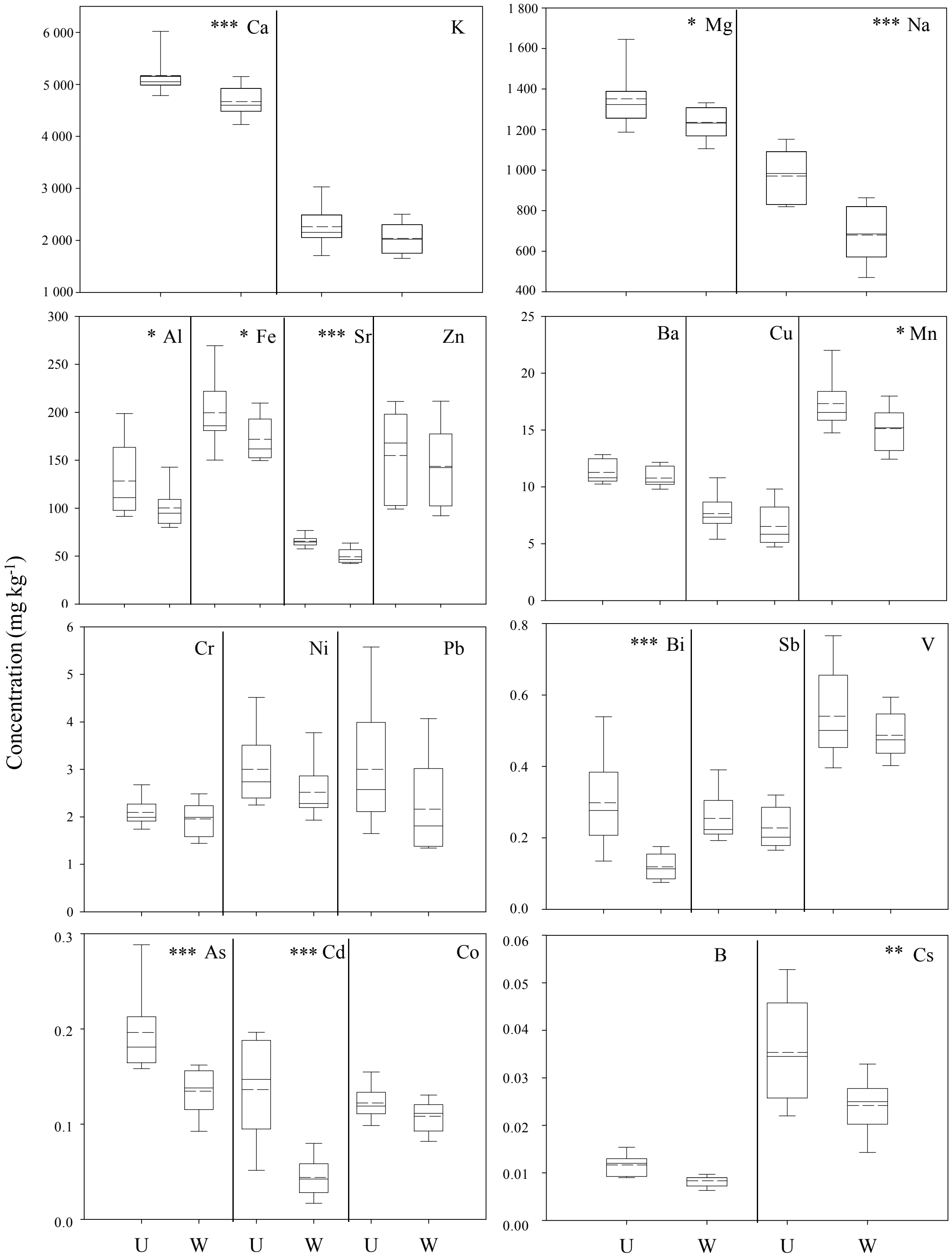

Figure 2. *Box–and–whiskers representation of the average content of selected trace elements in washed and unwashed samples of Tillandsia usneoides exposed from 6 May to 10 June, 2011 in the 4 stations of Pisa. For each element the top line represents the 90th percentile, the bottom line represents the 10th percentile and the box represents the 75th percentile (upper side), the 25th percentile (lower side) and the median (50th percentile, central line), respectively. Dashed line indicates the average concentration. For each element the significance following the t–test is shown: ***=p≤0.001; **=p≤0.01; *=p≤0.05. Please note the different scales on the y–coordinates.*

Table 1. *Mean concentrations (mg kg^{-1} dry weight) of selected trace elements in unwashed and washed samples of Tillandsia usneoides exposed from 6 May to 10 June, 2011 in the 4 sampling sites of Pisa compared with unwashed and washed samples in control site. Data are shown as mean±standard deviation*

Elements	Unwashed samples			Washed samples		
	Mean of sites	Control site	*p* value	Mean of sites	Control site	*p* value
Al	128±37	137±12	>0.05	100±20	102±16	>0.05
As	0.20±0.04	0.24±0.03	>0.05	0.14±0.01	0.18±0.03	>0.05
B	0.012±0.002	0.010±0.001	>0.05	0.008±0.001	0.005±0.001	>0.05
Ba	11.3±1.0	11.3±0.4	>0.05	10.8±0.9	9.7±0.2	>0.05
Bi	0.30±0.13	0.14±0.02	>0.05	0.12±0.04	0.09±0.02	>0.05
Ca	5 170±390	4 860±509	>0.05	4 669±297	4 804±153	>0.05
Cd	0.14±0.05	0.04±0.03	≤0.05	0.04±0.02	0.02±0.01	≤0.05
Co	0.12±0.01	0.10±0.01	≤0.05	0.11±0.02	0.08±0.01	≤0.05
Cr	2.7±0.1	2.7±0.4	>0.05	2.0±0.4	1.6±0.2	>0.05
Cs	0.035±0.011	0.025±0.001	>0.05	0.024±0.006	0.019±0.017	>0.05
Cu	7.7±1.7	6.6±0.6	>0.05	6.5±1.9	5.0±0.1	>0.05
Fe	200±38	188±12	>0.05	172±23	160±18	>0.05
K	2 264±404	2 353±152	>0.05	2 039±291	2 264±178	>0.05
Mg	1 352±146	1 375±54	>0.05	1 238±76	1 344±27	>0.05
Mn	17.3±2.3	16.5±0.7	>0.05	15.1±2.0	14.2±0.6	>0.05
Na	971±130	949±39	>0.05	680±137	756±59	>0.05
Ni	3.0±0.8	2.9±0.5	>0.05	2.5±0.6	2.1±0.3	>0.05
Pb	3.0±1.3	2.3±0.3	>0.05	2.2±1.0	2.3±0.3	>0.05
Sb	0.26±0.07	0.21±0.05	≤0.05	0.23±0.06	0.16±0.02	≤0.01
Sr	66±6	52±4	≤0.01	50±8	49±3	>0.05
V	0.54±0.13	0.43±0.03	>0.05	0.49±0.07	0.40±0.04	>0.05
Zn	155±46	90±16	≤0.05	144±41	89±9	≤0.05

A wide array of concentrations has been observed for most elements, suggesting that in our local monitoring network a clear location–specific differentiation regarding traffic intensity or other potential emission sources has played a role; this is even more clearly evident in Tables 2 and 3. Significant ($p \leq 0.05$) differences among sampling sites were observed for a wide group of elements [16 (unwashed) and 15 (washed) out of 22]. The concentrations of Al, Ba, Bi, Cd, Co, Cu, Pb, Sb, and Zn were the highest in UT and/or ST areas: some of these elements, notably Ba, Cu, Sb and Zn, are considered traffic–related elements (TREs) (Fujiwara et al., 2011) that involve many potential metal sources (Sternbeck et al., 2002). Ba has many applications in the automotive industries. Monaci and Bargagli (1997) found this element in samples of unleaded gasoline and diesel oil sold in Central Italy. So, presently Ba is regarded as the best inorganic tracer of vehicular traffic (see e.g. Sternbeck et al., 2002). Also Zn and Cu are directly related to the traffic density (Thorpe and Harrison, 2008).

Sb is ubiquitously present in the environment as a result of natural processes and human activities. In recent years, it has been associated with traffic and identified as a TRE (Paoli et al., 2012); in fact several parts of vehicles contain Sb alloys and other Sb compounds. In relation to its toxic properties and its dispersion in the environment, Sb is actually subjected to many pollution studies (Gueguen et al., 2012).

In I site, the main elements found were Mg, Sr and Zn. In relation to the first two, although Mg and Sr derived also from the soil, other important emission sources of this sampling site are various industrial processes. For this reason, it has been hypothesized that the presence of Mg could be related to a wide array of applications as a pH modifier and precipitant, filler, desiccant, absorbent and flocculant (like reported by Kainer, 2003); the presence of Sr could be related to the coal and oil combustion, incinerator ash and industrial wastes (such as confirmed by the ATSDR (2004). Zn could be originated from multiple sources: besides vehicular traffic, anthropogenic releases of Zn and its compounds to the atmosphere are from dust and fumes from zinc processing facilities and refuse incineration (ATSDR, 2005).

Fe, Mn, Na, and V concentrations were much higher in RR both in washed and unwashed samples. In relation to the first two, although Fe and Mn derived also from the soil, another important emission source of this sampling site is agricultural activity. For this reason, it has been hypothesized that the presence of Fe and Mn could be related to a wide array of agricultural applications as fertilizers and fungicides. Similar results were obtained by Bermudez et al. (2009) in three *Tillandsia* species collected from an agricultural area in the province of Cordoba (Argentina). Wannaz et al. (2006) suggested that Mn could be related to use of agrochemicals, such as fungicides. Although increased contents of Fe may be attributed to resuspended road dust (Zechmeister et al., 2006), in our case this element did not show any positive association with vehicular traffic. The evident presence of Fe in RR could be also associated with agricultural activities (especially use of fertilizers). Gimeno–Garcia et al. (1996) found high levels of Fe and Mn as impurities in fertilizers in Spain. Wannaz et al. (2006) found V associated with soil particles, although they hypothesized an anthropogenic origin for this element. Manta et al. (2002) supported a natural origin of V in urban soils of Italy. High Na concentrations associated with soil contamination of samples were found by Yenisoy–Karakas and Tuncel (2004) in the Aegean region of Turkey Regardless of differences among sites, B_{Vlocal} are exceeded 43 times (Table 2): 8 in ST (Al, Ba, Bi, Ca, Cd, Mg, Ni, and Sr), 13 in UT (Al, As, Ba, Bi, Co, Cu, K, Mg, Mn, Pb, Sb, Sr, and Zn), 11 in I (Bi, Ca, Cr, Cu, K, Mg, Na, Ni, Sb, Sr, and Zn) and 10 in RR (As, Ca, Cd, Co, Cr, Fe, Mn, Na, Ni, and V). The highest shift has been observed in ST (Bi) and UT (Pb). It should be noted that Zn is subject to long–range atmospheric transport, as found in previous studies (Bermudez et al., 2009). Rodriguez et al. (2010) reported an increase in Cu, Mn, Ni, Pb and Zn contents in *T. capillaris* exposed in Stuttgart (Germany) at a site with high levels of vehicular traffic. Figueiredo et al. (2004) observed that the highest concentrations of Cu, V and Zn were found in *T. usneoides* exposed for 8 weeks at stations of the São Paulo area (Brazil) rich in industrial and vehicular sources.

Inter–element relationships provide additional information on element sources (Table 4). TREs are significantly correlated among them [Cd vs. Zn ($p<0.001$, $R^2=0.79$), Cu vs. Sb ($p<0.01$, $R^2=0.53$), Cu vs. Zn ($p<0.05$, $R^2=0.40$), Pb vs. Zn ($p<0.01$, $R^2=0.40$)], in agreement with Manta et al. (2002). Vehicular traffic may thus well be the main emission source for these elements, as previously reported by Pignata et al. (2002) and by Klumpp et al. (2009). A significant correlation between lithogenic elements is verified in these case: Al vs. Cu (p<0.05, R^2=0.42), Ca vs Mg (p<0.05, R^2=0.40), Cu vs. Mg (p<0.05, R^2=0.40), Fe vs. Mn (p<0.001, R^2=0.76), Fe vs. K (p<0.01, R^2=0.40), K vs. Mg (p<0.01, R^2=0.51), K vs. Mn (p<0.05, R^2=0.40) and Mg vs. Mn (p<0.001, R^2=0.56). This supports the hypothesis that soil dust contributes largely to accumulation of fine particles on exposed Spanish moss samples in urban areas, in agreement with Adamo et al. (2008). The good correlation of Cr and V with Fe and Mn reflects their soil affinity (Manta et al., 2002).

Table 2. *Concentrations and local background (B_{Vlocal}) values (mg kg^{-1} dry weight) of selected trace elements in unwashed samples of Tillandsia usneoides exposed from 6 May to 10 June, 2011 in the 4 sampling sites of Pisa. Data are shown as mean±standard deviation. Within each row, different letters indicate significant differences (p≤0.05) among stations evaluated by one–way analysis of variance (ANOVA) and subsequent LSD post–hoc test. The mean concentrations of selected trace elements in control site are shown in Table 1*

Elements	Site				ANOVA	B_{Vlocal}
	Suburban/Traffic	Urban/Traffic	Industrial	Remote/Rural		
Al	185±31 c	145±21 bc	104±8 ab	101±10 a	≤0.01	123
As	0.18±0.02	0.20±0.04	0.17±0.01	0.23±0.07	>0.05	0.18
B	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	>0.05	0.01
Ba	12.0±0.9 b	12.5±0.1 b	10.7±0.2 a	10.5±0.2 a	≤0.05	11.3
Bi	0.48±0.08 c	0.30±0.03 b	0.26±0.03 b	0.16±0.04 a	≤0.001	0.24
Ca	5 069±146	4 920±220	5 622±596	5 068±31	>0.05	5 014
Cd	0.18±0.02 b	0.08±0.04 a	0.12±0.03 a	0.18±0.02 b	≤0.01	0.134
Co	0.11±0.01 a	0.15±0.02 b	0.11±0.01 a	0.16±0.02 b	≤0.01	0.13
Cr	1.8±0.1 a	2.0±0.1 ab	2.2±0.3 bc	2.4±0.3 c	≤0.05	2.0
Cs	0.03±0.01	0.02±0.01	0.03±0.01	0.03±0.02	>0.05	0.03
Cu	6.0±0.9 a	9.7±1.6 b	7.6±1.0 a	7.3±0.3 a	≤0.05	7.3
Fe	185±32 a	174±16 a	185±1 a	255±23 b	≤0.01	200
K	1 911±282	2 321±232	2 671±534	2 153±138	>0.05	2 186
Mg	1 326±67 ab	1 334±59 ab	1 525±189 b	1 223±51 a	≤0.05	1 296
Mn	15.6±1.3 a	16.7±0.7 a	16.3±0.4 a	20.7±1.9 b	≤0.01	16.4
Na	885±58 a	828±12 a	1 042±18 b	1 132±30 c	≤0.001	972
Ni	3.2±0.7	2.4±0.2	3.1±0.7	3.4±1.3	>0.05	3.0
Pb	2.4±0.1 ab	5.1±0.7 c	2.7±0.1 b	1.8±0.3 a	≤0.001	3.0
Sb	0.23±0.02 ab	0.34±0.11 c	0.30±0.06 bc	0.21±0.01 a	≤0.05	0.25
Sr	67±2 ab	65±3 ab	72±8 b	59±2 a	≤0.05	64
V	0.45±0.05 a	0.46±0.06 a	0.52±0.04 a	0.74±0.04 b	≤0.001	0.54
Zn	123±12 a	195±11 b	191±23 b	101±2 a	≤0.001	155

Table 3. *Concentrations (mg kg^{-1} dry weight) of selected trace elements in washed samples of Tillandsia usneoides exposed from 6 May to 10 June, 2011 in the 4 sampling sites of Pisa. Data are shown as mean±standard deviation. Within each row, different letters indicate significant differences (p≤0.05) among stations evaluated by one–way analysis of variance (ANOVA) and subsequent LSD post–hoc test. The mean concentrations of selected trace elements in control site are shown in Table 1*

Elements	Site				ANOVA
	Suburban/Traffic	Urban/Traffic	Industrial	Remote/Rural	
Al	90±13	123±24	102±11	86±8	>0.05
As	0.14±0.02	0.14±0.03	0.11±0.02	0.15±0.01	>0.05
B	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	>0.05
Ba	11.1±0.8 bc	12.0±0.3 c	10.3±0.1 ab	10.0±0.3 a	≤0.05
Bi	0.14±0.03 b	0.10±0.01 a	0.15±0.01 b	0.08±0.01a	≤0.01
Ca	4 789±386	4 374±202	4 943±108	4 570±55	>0.05
Cd	0.05±0.01 b	0.04±0.01 b	0.07±0.02 c	0.02±0.01 a	≤0.01
Co	0.09±0.01 a	0.12±0.01 c	0.10±0.01 b	0.13±0.01 c	≤0.001
Cr	1.5±0.1 a	1.9±0.3 b	2.0±0.1 b	2.4±0.1 c	≤0.01
Cs	0.03±0.01	0.02±0.02	0.03±0.01	0.03±0.01	>0.05
Cu	4.9±0.2 a	9.5±0.5 c	5.8±0.3 b	5.9±0.5 b	≤0.001
Fe	154±3 a	153±4 a	180±14 b	201±13 c	≤0.001
K	1 838±126	1 896±159	2 418±163	2 153±138	>0.05
Mg	1 263±64 bc	1 156±60 a	1 319±22 c	1 214±40 ab	≤0.05
Mn	13.1±0.9 a	13.8±0.9 a	14.2±1.6 b	17.4±0.9 b	≤0.001
Na	638±49 b	507±55 a	763±75 bc	811±88 c	≤0.01
Ni	2.9±0.1	2.1±0.2	2.3±0.1	2.8±1.1	>0.05
Pb	1.8±0.4 a	3.7±0.5 b	1.7±0.2 a	1.4±0.1 a	≤0.001
Sb	0.19±0.01 a	0.31±0.02 c	0.24±0.04 b	0.17±0.01 a	≤0.05
Sr	62±3 c	44±2 a	48±1 b	44±2 a	≤0.001
V	0.44±0.04 a	0.44±0.03 a	0.50±0.04 a	0.57±0.03 b	≤0.01
Zn	120±24 b	179±36 c	167±22 c	101±12 a	≤0.05

Table 4. *Matrix of determination coefficients (R^2) and P-values between elements in Tillandsia usneoides unwashed leaves exposed from 6 May to 10 June, 2011 in the 4 sampling sites of Pisa. Only values above 0.40 are shown*

	Al	As	B	Ba	Bi	Br	Ca	Cd	Co	Cr	Cs	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Sb	Sr	V	Zn
Al	-											0.42 ^{a}											
As		-			0.47 ^{b}																		
B			-																				
Ba				-									0.60 ^{c}	0.41 ^{a}		0.45 ^{b}	0.56 ^{b}		0.40 ^{a}			0.40 ^{b}	
Bi					-																		
Br						-																	
Ca							-							0.40 ^{a}	0.40 ^{a}								
Cd								-	0.40 ^{a}						0.40 ^{a}								0.79 ^{c}
Co									-	0.53 ^{b}											0.67 ^{c}		
Cr										-			0.53 ^{b}			0.64 ^{b}					0.49 ^{b}	0.53 ^{b}	
Cs											-												
Cu												-			0.40 ^{a}		0.40 ^{a}		0.80 ^{c}	0.53 ^{b}	0.43 ^{b}		0.40 ^{a}
Fe													-	0.40 ^{a}		0.76 ^{c}	0.56 ^{b}		0.40 ^{a}			0.82 ^{c}	
K														-	0.51 ^{b}	0.40 ^{a}	0.52 ^{b}						
Mg															-								
Mn																-	0.56 ^{b}					0.77 ^{c}	
Na																	-		0.79 ^{c}			0.54 ^{b}	
Ni																		-					
Pb																			-			0.40 ^{a}	0.40 ^{a}
Sb																				-			
Sr																					-		
V																						-	
Zn																							-

^{a} *$p \leq 0.05$*
^{b} *$p \leq 0.01$*
^{c} *$p \leq 0.001$*

In order to have an indication of the relative contribution of crustal contamination to the bulk of element distribution in *T. usneoides* samples, *EF*s were calculated for each element (Figure 3). Highly enriched elements were Cd, Sb and Zn (30%, 30% and 100% of sampling sites have *EF*s over 1 000, respectively), suggesting that their atmospheric concentrations are primarily due to non–crustal sources. As, Ba, Ca, Cr, K, Mg, Na, Ni, Pb, and Sr were considered enriched, with *EF*s in the range 10–100. *EF*s up to 10 (observed for B, Bi, Co, Cs, Cu, Fe, Mn and V) may be considered indicative of samples not significantly enriched. For As, Ca, Ni, Pb and Sr, *EF*s of most sampling sites exceeded 100 (respectively 25%, 92%, 42%, 42% and 73%). These results are in line with those reported by other authors (e.g. Isaac–Olive et al., 2012).

FA with Varimax rotation was applied to the dataset of total concentrations (Table 5). Eleven elements were included in the analysis. Three interpretable factors explained 95.4% of the total variance. The first one was characterized by high loadings for Fe and Mn and may be identified as a predominant soil contribution and accounts for 41.2% of the total variance. Other related elements are V [that could depend upon the parent material and the pedogenic process associated with its development (Krishna and Govil, 2004)], Mg and Sr. On the basis of *EF*s, these latter elements are considered enriched: this could indicate that even though the soil particles are providing part of these elements, their origin could be anthropogenic. The second factor (which explains 27.2% of the total variance) is loaded with Al, Ba and Sb: as already reported, these elements have been related to vehicular traffic. In the experimental study of Demiray et al. (2012), the results of cluster analysis in the *Xanthoria parietina* samples revealed the cluster 2 included two subgroups (2a: Al, Zn and Pb, 2b: Ti, As, Cd) suggesting that heavy traffic and road dust were one of the possible sources of these elements in an industrial district of Kocaeli province (Turkey). The results of principal component and cluster analyses for elements in *Sphagnum girgensohnii* bags and bulk deposits conducted by Anicic et al. (2009), showed that the first factor, containing the variables Al, Cr, Fe, Ni, and As, resuspended road dust. Finally, the third factor explains 27.0% of the total variance and is loaded with Cd, Cu and Zn. Since these elements can be associated with industrial and traffic variables, showing important contribution by urban emissions and traffic (as above–mentioned), we identified these elements as "tracers of anthropogenic point sources".

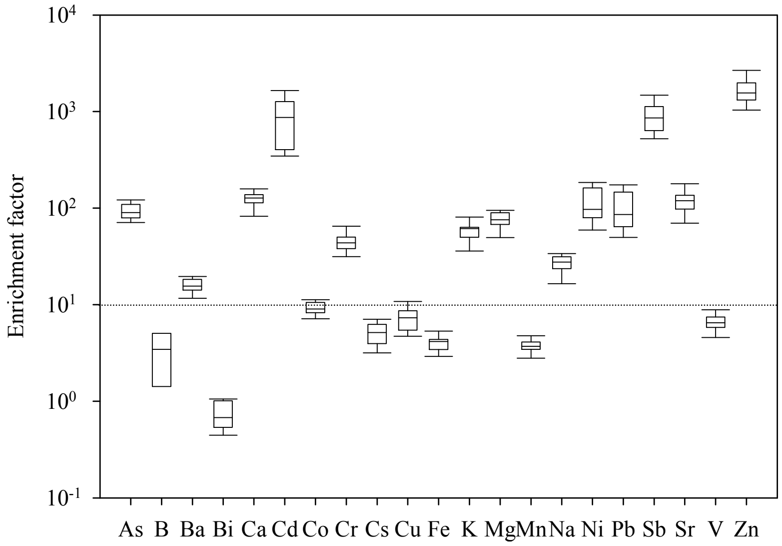

Figure 3. *Box and whiskers representation of crustal enrichment factors for elements in unwashed Tillandsia usneoides tissues exposed from 6 May to 10 June, 2011 in the 4 stations of Pisa. Al is the reference element. Please note the logarithmic scale of the ordinate axis. The dotted line represents the threshold value of 10. For each element, the top line represents the 90th percentile, the bottom line represents the 10th percentile and the box represents the 75th percentile (upper side), the 25th percentile (lower side) and the median (50th percentile, central line), respectively.*

Table 5. *Varimax rotation of principal factor pattern for elements in Tillandsia usneoides unwashed leaves exposed from 6 May to 10 June, 2011 in the 4 sampling sites of Pisa*

Parameter	Factor 1	Factor 2	Factor 3	Communalities
Al		–0.92		0.85
Ba		–0.92		0.85
Cd			0.90	0.83
Cu			–0.91	0.83
Fe	–0.88			0.78
Mg	0.60			0.36
Mn	–0.86			0.74
Sb		0.45		0.20
Sr	0.62			0.39
V	–0.86			0.73
Zn			–0.75	0.55
Eigenvalues	3.54	2.33	2.31	
% total var.	41.2	27.2	27.0	
Source type	Soil	Traffic and road dust	Anthropogenic point source	

4. Conclusive Remarks

As summarized by Markert (2008), a bioindicator is an organism that contains information on the quality of the environment, while a biomonitor is an organism that contains information on the quantitative aspects of the quality of the environment. In this light, it has been verified that the Spanish moss has: (i) high resistance/tolerance to heavy metal toxicity, (ii) specificity, (iii) capability to well–definitely represent a sampling site, (iv) quantitative response to pollutant exposure. Our results allow to confirm (i) and (ii), partly already verified (e.g. Figueredo et al., 2007). As far as characteristic (iii), the results of ANOVA indicated that the Spanish moss is able to reflect differences among the several areas and FA highlighted that it is also possible to trace the different source loadings. In summary, *T. usneoides* confirmed to be a suitable biomonitor, useful to highlight and map the concentrations of airborne trace elements in urban areas of the Mediterranean basin.

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