Airborne trace element pollution in 11 European cities assessed by exposure of standardised ryegrass cultures

Andreas Klumpp a,*, Wolfgang Ansel a, Gabriele Klumpp a, Jörn Breuer b, Philippe Vergne c, María José Sanz d, Stine Rasmussen e, Helge Ro-Poulsen e, Àngela Ribas Artola f,1, Josep Peñuelas f, Shang He g,2, Jean Pierre Garrec g, Vicent Calatayud d

a Institute of Landscape & Plant Ecology and Life Science Center, University of Hohenheim, 70599 Stuttgart, Germany
b State Institute of Agricultural Chemistry, University of Hohenheim, 70599 Stuttgart, Germany
c ENS Lyon and Lyon Botanical Garden, 46 Allée d’Italie, 69364 Lyon Cedex 07, France
d Fundación CEAM, Parque Tecnológico, c/ Charles Darwin 14, 46980 Paterna (Valencia), Spain
e Botanical Institute, University of Copenhagen, Øster Farimagsgade 2D, 1353 Copenhagen K, Denmark
f Unitat d’Ecofisiologia i Canvi Global CSIC-CEAB-CREATEF, CReAF (Centre de Recerca Ecològica i Aplicacions Forestals), Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain
g INRA Nancy, Laboratoire Pollution Atmosphérique, 54280 Champenoux, France

**Corresponding author. Tel.: +49 711 45923043; fax: +49 711 45923044.
E-mail address: aklumpp@uni-hohenheim.de (A. Klumpp).
1 Present address: Laboratory of Plant Ecology and Forest Botany, CTFC – Technological Forest Centre of Catalonia, Pujada del Seminari s/n, 25280 Solsola, Spain.
2 Present address: Chinese Academy of Forestry, Research Institute of Forest Ecology and Environmental Science, Wan Shou Shan, Beijing 100091, PR China.

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1. Introduction

Ambient levels of particulate matter (PM) in urban areas have recently gained in importance and are now in the focus of public and political discussions in Europe. This increased interest is a result of the fact that the new European PM10 threshold values (EU, 1999) that have been enforced since 2005 are frequently being exceeded in
many urban centres throughout Europe, forcing environmental authorities to adopt action plans and to take mitigation measures such as the establishment of low emission zones. The more stringent threshold value has been motivated by the severe effects of PM on human health. Premature deaths of about 350,000 people annually are being attributed to exposure to current particle levels in Europe (EEA, 2005). As a consequence, the strong health risk caused by PM, especially PM$_{2.5}$, will be addressed under the revised European air quality legislation (EU, 2008). PM is known to exert direct effects on human health, particularly by affecting the respiratory system, resulting in increased morbidity and mortality rates. In addition to physical aspects such as particle number, size, and surface area, the chemical composition determines the direct or indirect toxic and genotoxic effects of PM after uptake through inhalation or ingestion (EEA, 2005; WHO, 2003). Elemental carbon and inorganic anions like sulphate and nitrate seem to be the main constituents of PM$_{10}$ and PM$_{2.5}$ (Putaud et al., 2004), but trace metals may also play a major role concerning toxicity and ecotoxicity of dust particles and their adverse health effects (Götschi et al., 2005; Valavanidis et al., 2006). This importance can be explained by the observation that metals have a high affinity to the very fine and ultrafine aerosol fractions (Moreno et al., 2006).

The air quality strategy of the European Union is based on physico-chemical measurements of ambient pollution concentrations and complementary modelling techniques. The Air Quality Framework Directive (EU, 1996) and so-called Daughter Directives have been introduced to establish air quality standards (limit and target values, respectively) for the major groups of air pollutants, and measurement programmes to monitor pollutant concentrations in the air. The most recent daughter “directive relating to arsenic, cadmium, mercury, nickel and polycyclic aromatic hydrocarbons in ambient air” (EU, 2004), however, recommends that, in addition to mandatory measurements of atmospheric pollutant concentrations, “the use of bioindicators may be considered where regional patterns of the impact on ecosystems are to be assessed.”

Biomonitoring of trace element pollution is normally conducted by using accumulative bioindicator plants, since atmospheric levels of heavy metals and other trace elements generally do not cause visible injury symptoms. Regional and even Europe-wide patterns of heavy metal impact have frequently been assessed by passive monitoring using epiphytic lichens and terrestrial mosses. In some cases, transplantation techniques (i.e., active monitoring) have also been applied, e.g., by exposure of lichen transplants along roads or in urban and industrialised areas (Conti and Cecchetti, 2001; Garty, 2001; Godinho et al., 2008; and many others). In Germany, cultures of Italian ryegrass (Lolium multiflorum Lam. ssp. italicum cv. Lema) have been used as accumulative bioindicators of trace elements, sulphur, fluoride, and organic pollutants since the early 1970s. This species presents a high capacity for the accumulation of toxic substances, as well as a high tolerance against most air pollutants, without showing any visible injury due to ambient pollution levels. Being a commonly grown fodder plant, the accumulation of toxic substances in ryegrass may serve for estimating the potential biomagnification of contaminants along the food chain and the potential health risk for humans and livestock. A standardised method describing all steps, from cultivation to field exposure and sampling, was established and published as a guideline of the Association of German Engineers (VDI) as early as 1978. This procedure has since been used extensively in Germany to assess the accumulation of toxic substances in regional and local surveys, along major roads and around industrial plants (Dietl et al., 1996, 1997; Franzaring et al., 2007; Nobel et al., 2004). Similar methods using Italian ryegrass, perennial ryegrass, or other pasture grass species have also been applied in biomonitoring studies in several European countries, e.g., in Austria (Öhlinger and Döberl, 1992; Stabentheiner et al., 2004), Belgium (De Temmerman and Baeten, 1988; De Temmerman et al., 2007), Italy (Caggiano et al., 2001), Spain (Rey-Asensio and Carballeira, 2007), and even in South America (Domíngos et al., 1998; Klumpp et al., 1994). A completely revised and updated guideline for standardised exposure of grass cultures was published some years ago (VDI, 2003), and will now serve as a reference document for elaborating a European standard procedure by the European Committee for Standardization (CEN).

The present paper reports on the use of the standardised grass culture method for the assessment of airborne trace element pollution in European cities. This study was part of the major pan-European biomonitoring programme EuroBionet, which applied several standardised bioindication methods in urban agglomerations of eight EU Member States during the period from 1999 to 2002 (Klumpp et al., 2002, 2004). It aimed to assess the temporal and spatial variability of airborne trace element pollution at urban, suburban, traffic-exposed and rural sites, and to test the suitability of the chosen method for biomonitoring of trace elements in various European regions. Our paper focuses on the accumulation of ten elements (As, Cd, Cr, Cu, Fe, Ni, Pb, Sb, V, Zn) in grass cultures exposed to ambient air at up to 100 monitoring stations established in 11 local networks in 2001. Comparisons of trace metal contents in grass culture with limit values for animal feed and for foodstuffs were made to estimate potential health risks. More specific information on single monitoring networks is given by Klumpp et al. (2004).

2. Material and methods

2.1. The EuroBionet programme

The European bioindicator programme EuroBionet was conducted in urban agglomerations in eight EU Member States (Klumpp et al., 2002, 2004) under the coordination of the University of Hohenheim (UHOH). The project started with municipalities and research institutes from the following cities and regions as participants: Copenhagen (Denmark), Edinburgh (UK), Klagenfurt (Austria), Greater Lyon (France), Sheffield (UK), and Verona (Italy). The City of Düsseldorf (Germany), the City of Ditzingen/Greater Stuttgart (Germany), Greater Nancy (France), and the regional
government of Catalonia/Barcelona (Spain) joined the network in 2000, and the cities of Valencia (Spain) and Glyfada/Greater Athens (Greece) joined in 2001. For more information on EuroBionet refer to the project website (www.eurobionet.com).

2.2. Local networks

In each city, local bioindicator networks consisting of eight to ten exposure sites were implemented, including one or two reference sites with low levels of primary air pollutants, as well as urban, suburban, industrial, and traffic-exposed sites. Overall, about 100 bioindicator stations were established and operated over up to three years. Various criteria were considered when selecting the monitoring sites. The first was a relatively uniform distribution over the city area to best represent the pollution burden of the conurbation. Proximity to existing air monitoring stations, protection from theft and vandalism, and city planning matters also played an important role. The 'Stuttgart' monitoring network did not include bio-monitoring sites in the city centre but three sites in the network’s associate partner, the township of Ditzingen northwest of the Stuttgart/Middle Neckar conurbation, as well as sites on the university campus and in three municipalities in the Neckar valley southeast of Stuttgart. They were run directly by the coordination team and were treated as one network. At all the sites, various bioindicator species were exposed to ambient air in order to assess the effects of ozone, sulphurous compounds, trace elements, hydrocarbons, and mutagenic substances (Klumpp et al., 2002, 2004, 2006).

2.3. Cultivation and field exposure of grass cultures

Seeds of Italian ryegrass (*Lolium multiflorum* Lam. cv. “Lema”) were obtained from Norddeutsche Pflanzenzucht (Holtsee, Germany) and distributed to the local teams. The cultivation of grass cultures was performed according to a guideline published by VDI (2003) in the greenhouses of the participating cities using plastic pots (1.3 L) and commercially available, non-fertilised standardised soil (Type 0; Patzer Einheitserde). Two glass fibre wicks (Ø 5 mm) were inserted through holes in the bottom of each pot. 0.6 g of seeds were sown directly on the soil surface and then covered by a 3-mm-thick layer of sifted soil. The soil surface was moistened by spraying with deionised water, and pots were placed upon water-filled basins so that the wicks were hanging into the water, thus supplying the grass cultures with water. Drying during the germination phase was avoided by occasional spraying with water. The grass cultures were regularly fertilised with NPK solution made from analytic-grade chemicals. When the blades reached a height of 8–10 cm, the grass cultures were cut back to a stubble height of 4 cm with a pair of ceramic-coated scissors to stimulate tillering. Further cut-backs were made every 8–10 days during cultivation and finally one day before start of field exposure.

After greenhouse cultivation for approx. 6 weeks, the grass cultures were exposed to ambient air at the

![Fig. 1. Exposure device for ryegrass biomonitoring (left: diagram showing the water storage container with overflow hole and the plant pot with inserted glass fibre wicks; right: view of an exposure station).](image-url)
After an exposure of 28 ± 1 days, the grass cultures were harvested, i.e., the plant material grown during the exposure period was cut at 4 cm height. Samples with less than 2 g dry weight (DW) were rejected to avoid artefacts due to irregular growing conditions during single exposure periods. After sampling, the cultures were discarded and replaced with new ones. The air-dried plant samples were dispatched for analysis to the central lab at UHOH. Up to five consecutive exposure periods were performed between mid May and mid September every year. Exposure of grass cultures in the different local networks was synchronised, i.e., exposure periods in the different cities and regions generally started and ended on the same day, thus facilitating later comparison among networks. As Glyfada did not join the network until late 2001, data from ryegrass exposure is only available for 2002 and was therefore not included in this paper.

2.4. Chemical analyses

All chemical analyses were performed at the central labs at UHOH. The samples were dried to constant weight at 80 °C (weight loss < 80%) and ground using an agate mill. For determination of metal contents, a wet digestion (10 mL HNO3/10 mL H2O/dest./3 mL H2O2/1 drop HF per 500 mg ground plant material) was performed in a microwave pressure digestion system (MLS-Ethos Plus). Trace element analyses were done using an atomic absorption spectrometer (Varian SpectrAA-220/GTA-110). For the atomisation of iron (Fe), copper (Cu), and zinc (Zn), an air/acetylene flame was used whereas an electrothermal heated graphite furnace was used for lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni). Antimony (Sb), arsenic (As), and vanadium (V) were determined using an inductively-coupled plasma mass-spectrometer (ICP-MS, Elan 6000, Perkin Elmer Sciex). The ICP-MS was equipped with a standard Scott-type spray chamber and a cross-flow nebuliser. Rhodium (10 µg L⁻¹) was added to all standard and sample solutions as an internal standard to correct for drift of the instrument. The certified reference materials CRM 281 (‘ryegrass’, European Commission/BCR) and SRM 1515 (‘apple leaves’, National Institute of Standards and Technology/NIST) served as standards for quality control of analyses, and the recovery rate was usually >95%.

2.5. Evaluation of data and statistical methods

For evaluation of data, a method developed by Erhardt et al. (1996) and adopted by VDI (2003) was applied which permits one to calculate process-inherent background values (Bv) and threshold values (i.e., effect detection limits EDL). In this iterative procedure, the numerous low values in a study area, which indicate only a low pollution impact, are used as reference values. First, mean site values and standard deviation were calculated for each local network and each exposure series. Subsequently, single values exceeding a filter threshold (Fj) defined as mean site value (xj) plus 1.96 times the standard deviation sd – (Fj = xj ± 1.96 sd) – were removed from the data collective and the new mean value 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 (Bvlocal). In a similar way, a European background value BvEurope was calculated by joining all reference values from the local networks. The threshold for pollution-induced accumulation of elements (i.e., EDL) was defined as mean background value plus the threefold standard error (Erhardt et al., 1996).

For graphic presentation of data, box–whisker plots were drawn for each element and each experimental year. Differences between local networks were evaluated by analysis of variance (ANOVA) and subsequent Least Significant Difference (LSD) test. Relationships between the contents of individual elements in grass samples were tested using Spearman rank correlation coefficients. With cluster analysis using squared Euclidean distance as a measurement of distance and the Ward algorithm as an agglomeration procedure, the monitoring points were subclassified into groups (“clusters”) according to their relative similarity.

2.6. Quality assurance and control

The project placed special emphasis on the standardisation of all procedural steps, including plant cultivation and exposure at the monitoring sites, sampling of plant material, analytical methods, data acquisition and processing (see also previous sections). This strict harmonisation was designed to eliminate potential external factors that could influence plant response and to reduce methodological error. More details on the quality assurance and control measures were given by Klumpp et al. (2006).

3. Results

3.1. Trace element concentrations in ryegrass cultures

Table 1 gives the mean background values of trace elements in standardised ryegrass cultures exposed in the local monitoring networks during five periods of four weeks each in 2001, the mean European background value, and the effect detection limits for the same study year. For comparison, data determined in biomonitoring programmes conducted in Southern Germany during the same period of time (VDI, 2003) is also presented. Within the EuroBionet network, smaller cities like Klagenfurt, Nancy and Verona, and the more suburban/rural monitoring network in the Stuttgart region, were characterised by very low to low background levels for the majority of the elements analysed. Comparatively low background values were also found in Edinburgh, Lyon, and Copenhagen. Higher metal levels, by contrast, were detected in some larger conurbations like Sheffield and Düsseldorf, and particularly in the two Spanish cities Barcelona and Valencia. The latter two networks presented the highest
background levels for nine out of ten elements analysed in the present study. Pb and Sb background levels, e.g., in Valencia were 5.8 and 4.9 times higher than the European background, and the V background in Barcelona was even 6.8 times the B \text{vEur}. Only Cd levels in Spanish cities were somewhat lower than in several other networks. A comparison of background values for the study years 2000 (data not shown) and 2001 revealed an extensive consistency for the local as well as the European background values in both years.

A detailed overview of the concentrations of individual trace elements and their variability within the local monitoring networks is provided in the box–whisker plots in Fig. 2. Pollution with Cd (Fig. 2a) was relatively low throughout the whole EuroBionet monitoring network when compared to other data (VDI, 2003), with somewhat higher levels in the bigger cities. Despite the comparatively large scattering of the concentrations in our local monitoring networks, no clear location-specific differentiation regarding traffic intensity or other potential emission sources was observed.

For another group of elements comprising As, Ni, and Zn, only a small scattering of the values was usually found within the monitoring networks, and in most cases the maximum site values exceeded background levels only by a little (Fig. 2b–d). With Ni, the very small scattering between the individual bioindicator stations was particularly striking (Fig. 2b), just one site in the Sheffield monitoring network was much above the overall pollution level. In the Barcelona network, much higher As concentrations were measured compared to the remaining cities (Fig. 2c). The Zn contents, like Ni, were distributed relatively evenly over the entire European monitoring network. Only in the Spanish cities were slightly higher values and larger ranges recorded (Fig. 2d).

The majority of the V concentrations lay on a relatively uniform, low level, and clearly raised values appeared only occasionally (Fig. 2e). The high pollution of the two Spanish cities was also obvious for this element. There, even the minimum site means of the local reference stations were clearly above most of the maximum site means for the other cities of the network. In light of these large differences, it can largely be ruled out that the make-up of the individual monitoring networks, i.e., the number of more heavily polluted traffic and city centre stations on one side and suburban and reference stations on the other side, has influenced the results significantly.

An entirely different behaviour compared to the trace elements presented above was shown by Sb, Pb, Cr, Fe, and Cu. With these elements, the large scattering within individual local monitoring networks and the large ranges between minimum and maximum values, especially in the bigger cities, were particularly striking features of the box–whisker plots (Fig. 2f–j). Thus, values for mean minimum and maximum values varied for Cu in Copenhagen by a factor of about 4, for Pb in Barcelona and Valencia by

Table 1

<table>
<thead>
<tr>
<th>City *</th>
<th>As</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Ni</th>
<th>Pb</th>
<th>Sb</th>
<th>V</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ed</td>
<td>0.45</td>
<td>0.05</td>
<td>0.7</td>
<td>8.2</td>
<td>219</td>
<td>6.5</td>
<td>0.8</td>
<td>0.15</td>
<td>0.43</td>
<td>32.1</td>
</tr>
<tr>
<td>Sh</td>
<td>0.21</td>
<td>0.10</td>
<td>2.2</td>
<td>8.2</td>
<td>224</td>
<td>6.2</td>
<td>2.0</td>
<td>0.25</td>
<td>0.31</td>
<td>33.1</td>
</tr>
<tr>
<td>Co</td>
<td>0.47</td>
<td>0.06</td>
<td>0.6</td>
<td>6.2</td>
<td>145</td>
<td>4.2</td>
<td>0.9</td>
<td>0.37</td>
<td>0.32</td>
<td>24.8</td>
</tr>
<tr>
<td>Dü</td>
<td>0.52</td>
<td>0.09</td>
<td>1.7</td>
<td>9.0</td>
<td>223</td>
<td>7.1</td>
<td>1.4</td>
<td>0.31</td>
<td>0.37</td>
<td>38.6</td>
</tr>
<tr>
<td>Na</td>
<td>0.33</td>
<td>0.04</td>
<td>0.8</td>
<td>7.9</td>
<td>216</td>
<td>5.3</td>
<td>0.9</td>
<td>0.19</td>
<td>0.37</td>
<td>31.6</td>
</tr>
<tr>
<td>St</td>
<td>0.41</td>
<td>0.04</td>
<td>0.5</td>
<td>7.5</td>
<td>157</td>
<td>4.8</td>
<td>0.6</td>
<td>0.26</td>
<td>0.31</td>
<td>33.6</td>
</tr>
<tr>
<td>Kl</td>
<td>0.39</td>
<td>0.03</td>
<td>1.0</td>
<td>4.5</td>
<td>229</td>
<td>5.4</td>
<td>0.5</td>
<td>0.12</td>
<td>0.56</td>
<td>32.7</td>
</tr>
<tr>
<td>Ve</td>
<td>n.d.</td>
<td>0.04</td>
<td>0.7</td>
<td>8.1</td>
<td>162</td>
<td>5.9</td>
<td>0.9</td>
<td>0.20</td>
<td>0.35</td>
<td>28.2</td>
</tr>
<tr>
<td>Ly</td>
<td>0.59</td>
<td>0.07</td>
<td>1.1</td>
<td>6.0</td>
<td>191</td>
<td>3.7</td>
<td>1.5</td>
<td>0.35</td>
<td>0.33</td>
<td>31.4</td>
</tr>
<tr>
<td>Ba</td>
<td>0.76</td>
<td>0.05</td>
<td>3.1</td>
<td>14.1</td>
<td>628</td>
<td>7.3</td>
<td>3.3</td>
<td>0.59</td>
<td>2.17</td>
<td>50.8</td>
</tr>
<tr>
<td>Va</td>
<td>0.23</td>
<td>0.06</td>
<td>1.7</td>
<td>9.8</td>
<td>476</td>
<td>3.8</td>
<td>4.6</td>
<td>0.83</td>
<td>1.81</td>
<td>38.2</td>
</tr>
</tbody>
</table>

| BvEur | 0.38| 0.04| 0.8 | 7.1 | 180 | 5.5 | 0.8 | 0.17| 0.32| 31.7|
| EDL   | 0.40| 0.05| 0.9 | 7.7 | 197 | 6.0 | 0.9 | 0.20| 0.35| 33.3|

| BvVDI | -0.28| 0.07| -0.6 | -12.4| 135 | -13.7| -1.2 | -0.05| 0.13| -43.0|
| EDLVDI| 0.24| 0.08| 0.7 | 7.0 | 6.4 | 0.5 | 0.05| 35.0|

| -0.31| -0.10| -0.9 | -13.7| 153 | -15.4| -1.6 | -0.06| 0.16| -61.0|

* Ed = Edinburgh, Sh = Sheffield, Co = Copenhagen, Dü = Düsseldorf, Na = Nancy, St = Stuttgart, Kl = Klagenfurt, Ve = Verona, Ly = Lyon, Ba = Barcelona, Va = Valencia.
a factor of 5, for Fe in Copenhagen and Barcelona by a factor of 4, and for Sb in Barcelona, Copenhagen, and Valencia by a factor of 10. A more detailed assessment of the local monitoring networks showed that the highest concentrations of these elements occurred (except for Cr in Sheffield) at sites very heavily burdened by traffic in cities with a large number of inhabitants. The accumulation of trace elements was especially strong when the stations had been set up close to roadsides: examples include the sites Radhuspladsen in Copenhagen, Mörsenbroicher Ei and Graf-Adolf-Platz in Düsseldorf, as well as Badalona in Barcelona and Avenida Aragón in Valencia.

Overall, it can be stated that the smaller cities of the network, especially Nancy, Klagenfurt, Verona, and the rather more rural than urban Stuttgart network, featured relatively low levels of trace element pollution, with most values being close to effect detection limits and background values. Larger cities like Edinburgh, Sheffield, Copenhagen, Düsseldorf, Lyon, Barcelona, and Valencia, by contrast, generally showed higher levels of trace element accumulation and greater variation among individual sites.

3.2. Source apportionment

The Spearman’s rank correlation analysis revealed numerous significant correlations between most of the metals analysed (Fig. 3). The contents of Sb, which arises primarily from the abrasion of vehicle brake linings, were closely correlated with Pb, Cr, Fe, and Cu concentrations. As such, motor vehicle traffic may therefore represent the main emission source for this group of pollutants. A close correlation was evident between Fe and V, which are released during the combustion of heavy-duty fuel oil in...
power generation. Comparably high correlation coefficients were also found with the plant nutrients Cu and Zn, as well as Cu and Ni.

Cluster analysis was used for separating the monitoring stations into groups (clusters) with high degrees of similarity regarding the concentrations of trace elements accumulated in the grass cultures. Upon use of the variables Sb, Pb, Cr, Fe, and Cu, i.e., the elements presumed to be emitted primarily by car traffic, four clusters could be distinguished (Table 2). The first cluster included the sites established at the major traffic arteries of the Spanish cities Barcelona and Valencia. Cluster 2 was formed from a single site in Sheffield, further street sites in other cities of the network as well as the remaining Spanish monitoring sites (apart from the reference stations) were grouped into the third cluster. Even when including all heavy metals as variables in the analysis, no other cluster distribution was obtained.

## 4. Discussion

### 4.1. Comparison with other biomonitoring programmes

In general, there was a good agreement between the background values and effect detection limits determined in the present study and those of other biomonitoring projects (VDI, 2003). However, it should be mentioned here that in our study, sensu strictu, the calculation of background values, and thus a comparison with data from the studies summarised by VDI (2003), is not correct when strictly applying the rules established by Erhardt et al. (1996). According to these authors, the calculation of such background values presupposes that the majority of monitoring sites in a given network are actually background sites without direct influence of specific emission sources. This was not the case in our networks, which focused on polluted sites in city centres and included only a small number of less polluted suburban and rural sites. However, even subject to these restrictions, the comparison shows that the trace element concentrations in ryegrass in our investigations are similar to data from other studies conducted in the same period of time. The results also underline that the observed variability of heavy metal levels in different cities is not, or is only partly, a result of differences in network set-up.

The results of trace element analyses were compared among the 11 local monitoring networks of our study, but also with data from several biomonitoring studies with grass cultures conducted in urban areas and along major roads in Germany and Austria, using the same methodology since the early 1990s (Dietl et al., 1996, 1997; Erhardt et al., 1996; Köhler and Peichl, 2002; Nobel et al., 2004; Peichl et al., 1994; Stabentheiner et al., 2004; VDI, 2003; Wäber et al., 1998). The comparison revealed that the Cd levels in our study were indeed quite low, indicating lower environmental Cd loads than in former years. This observation was corroborated by Nobel et al. (2004), who reported on a clear downward trend of Cd contents in ryegrass during repeated exposure experiments conducted between 1997 and 2000. They found the average Cd levels declining from 0.6 μg g⁻¹ DW in 1997 to 0.1 μg g⁻¹ DW in 2000. Ni and Zn concentrations in our study, by contrast, were quite similar to the results obtained in an Austrian urban monitoring network in 1996 (Stabentheiner et al., 2004) whereas the As levels were clearly higher than those from the Austrian network. As V

### Table 2

Results of the cluster analysis for the elemental group Cr, Cu, Fe, Ni, Pb, and Sb, i.e. those elements supposedly emitted by car traffic.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Site characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>Three street sites in the two Spanish cities Barcelona (1 site) and Valencia (2 sites)</td>
</tr>
<tr>
<td>Cluster 2</td>
<td>One industrial and traffic-influenced site in Sheffield</td>
</tr>
<tr>
<td>Cluster 3</td>
<td>Monitoring stations next to main traffic arteries in Edinburgh (1 site), Copenhagen (1 site) and Düsseldorf (2 sites) as well as all Spanish stations (Barcelona: 5 sites, Valencia: 5 sites) except the two reference sites and the three sites forming cluster 1</td>
</tr>
<tr>
<td>Cluster 4</td>
<td>All remaining sites</td>
</tr>
</tbody>
</table>

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Fig. 3. Results of the Spearman rank correlation analysis for trace element concentrations in grass cultures in 2001 (n = 95, r = correlation coefficient, p = error probability, one-tailed significance). Significant correlations are marked in yellow (**: p < 0.01, dark grey (**: p < 0.05), and light grey (*: p < 0.05).
accumulation is rarely determined in biomonitoring studies, the database was not sufficient for comparison with other networks.

The association of the trace metals Sb, Pb, Cr, Fe, and Cu with emissions from vehicular traffic shown by cluster analysis has been demonstrated previously in various biomonitoring programmes (e.g., Dietl et al., 1997; Köhler and Peichl, 2002; Nobel et al., 2004). Correspondingly, Sb levels at traffic-exposed sites in the present study compared very well to data from grass cultures exposed close to a highway and in a city centre in S-Germany (Dietl et al., 1996, 1997; Nobel et al., 2004; Wäber et al., 1998). The maximum values obtained in some major cities of our network, however, exceeded clearly the Sb contents of the other studies. The Pb levels in the EuroBionet programme were higher than those at a highway in Germany (Nobel et al., 2004), but similar to findings from other urban areas (Köhler and Peichl, 2002; Stabentheiner et al., 2004), except for the situation in the two Spanish networks where much higher values were found (see below). Environmental Pb levels, including concentrations in ambient air, dust particles, soil, and vegetation, have been strongly decreasing because of the phase-out of leaded petrol in Europe. This decline has also been demonstrated by long-term biomonitoring programmes using ryegrass and other bioindicator plants (Nobel et al., 2004; and others). Most sites of our study showed Cr, Cu, and Fe concentrations similar to the other biomonitoring programmes cited previously (Köhler and Peichl, 2002; Nobel et al., 2004; Stabentheiner et al., 2004). Besides the Spanish cities, the only other exception was a site in Sheffield (UK) with very strong accumulation of several trace elements, mainly Cr and Ni, which proved to be affected by emissions from a local steel works (Klumpp et al., 2004; Schelle et al., 2008).

4.2. Metal load in Spanish cities

When comparing the summarised results (Fig. 2) of trace element analyses among the city networks, the strong accumulation in grass samples from Spanish cities was particularly outstanding. With few exceptions, background levels as well as mean values and maximum site means of the Barcelona and Valencia networks were clearly higher than those of the other local networks. In the case of V, the minimum site means exceeded even the maximum values of the other cities. Unfortunately, no data from other ryegrass exposure studies in Spain are available for comparison.

However, from several regional and European studies on atmospheric concentrations and deposition of PM, it is known that urban agglomerations in Spain frequently feature high aerosol and heavy metal loads. In a pan-European measuring campaign on elemental composition of fine particles (PM$_{2.5}$) including 21 urban sites from Finland to Spain, the highest annual mean values for several trace elements were found in cities in Northern Italy and Spain. Barcelona figured among the three most polluted cities concerning As, Cu, Fe, Pb, V, and Zn (Götschi et al., 2005). Further European (Putaud et al., 2004; Sillanpää et al., 2006; Viana et al., 2007a) and regional (Moreno et al., 2006; Querol et al., 2007; Viana et al., 2007b) studies on chemical composition and mass closure of urban aerosols confirmed that Barcelona ranges among the cities with highest PM loads leading to frequent exceedances of limit values. Trace elements concentrations in tree leaves and mosses sampled in the Barcelona metropolitan region were much higher than in other Mediterranean urban areas (Sardans and Peñuelas, 2005). Elevated levels of fine and coarse dust in Barcelona were partly attributed to long-range transport of Saharan dust, low dispersive conditions and resuspension of crustal particles from the soil, but high concentrations of trace metals in dust fractions were ascribed to local anthropogenic emission sources like vehicle traffic and industrial plants (Moreno et al., 2006; Pérez et al., 2008; Viana et al., 2007b). High levels of Pb, Sb, Cu, and other traffic-related elements determined in dust samples from those studies, as well as in plant samples in our own study, reflect the strong contribution of vehicular traffic to the PM load in general, and especially to heavy metal pollution in Spanish cities. This is also supported by previous reports on the relatively high percentage of cars fuelled by leaded petrol which have been in operation in Spain until recently, and on the reduction of environmental Pb levels after the final ban of leaded petrol in 2001. Additionally, resuspension of dust and soil particles contaminated by former Pb emissions may play a major role under the dry climatic conditions of the Mediterranean summer. The latter effect may explain why ryegrass cultures showed a strong accumulation of other traffic-related metals like Cu, Cr, and Fe which are not associated with the use of Pb additives in petrol, particularly at the traffic-exposed sites in Spain. High V concentrations in bioindicator plants and in dust samples in Barcelona and probably also in Valencia, by contrast, can be traced back to the influence of heavy oil combustion in power plants, petrochemical works and shipping traffic in the harbour (Moreno et al., 2006; Viana et al., 2007b).

4.3. Assessment of potential health risks due to trace element deposition

Broadly accepted threshold and reference values for trace element concentrations in ryegrass or other bioindicator species are currently not available, but will be laid down in future VDI guidelines (Nobel et al., 2005). Meanwhile, limit values for animal feed and for foodstuffs fixed in European legislation (EU, 2002, 2005, 2006) as well as values for maximum intake dose (MID) recommended by VDI (1996–2007) may be used to evaluate data from biomonitoring studies and to assess the potential risk to animals and human health. For such comparison, however, it should be emphasized that limit values for foodstuff are generally expressed on a fresh weight (FW) and for feed on a 12% moisture content basis (DW$_{12}$).

As mentioned before, Cd levels in ryegrass were quite low in the whole EuroBionet. At all stations, the measured values were much lower than the applicable European thresholds for animal feed and foodstuffs, which are at 0.5–1.0 mg kg$^{-1}$ DW$_{12}$ for different feed materials (EU, 2005) and 0.1–0.2 mg kg$^{-1}$ FW for stem and leaf vegetables (EU, 2006), respectively. Likewise, although Ni contents were clearly elevated at some sites in Sheffield, Edinburgh, and Barcelona, even the maximum site mean of...
13.6 mg kg\(^{-1}\) DW did not approach the threshold value (MID) of 50 mg kg\(^{-1}\) DW\(_{12}\) recommended for different types of feed (VDI, 1996–2007). On the other hand, the distance between measured trace element contents in grass and the above mentioned legal limit values was quite a bit smaller for other elements. In the case of As, for instance, the maximum site mean of the most polluted site in the Barcelona network (1.2 mg kg\(^{-1}\)DW\(_{12}\)) reached about 50% of the threshold value of 2 mg kg\(^{-1}\)DW\(_{12}\) in animal feed (EU, 2002). The peak V values (up to 4.6 mg kg\(^{-1}\) DW) clearly exceeded the recommended MID values for feed for especially sensitive livestock (VDI, 1996–2007) at several stations in Barcelona, and also in Valencia.

As discussed earlier, the accumulation of traffic-related metals was stronger the closer to roadsides the monitoring sites had been set up. At several of these traffic-exposed sites, e.g., in Copenhagen, Düsseldorf, Barcelona, and Valencia, the maximum Cu intake values for sheep (10–20 mg kg\(^{-1}\) DW\(_{12}\)) recommended by VDI (1996–2007) were reached and sometimes clearly exceeded. Although Pb contents were in most cities much lower than those measured in other conurbations in earlier studies, because of the phase-out of leaded petrol, the Pb contamination of grass remained relatively high when compared to limit values. Particularly in the two Spanish cities, the situation in 2001 was still very different from the other cities because of the delay in the introduction of unleaded fuels. At many sites in Barcelona and Valencia, Pb concentrations in ryegrass reached nearly 50% of the threshold of 30 mg kg\(^{-1}\) DW\(_{12}\) for green fodder, and exceeded the much lower limit values concerning feeding stuff for other types of livestock (EU, 2005; VDI, 1996–2007). Applying the European regulation for foodstuffs, which prescribes a maximum Pb level in leafy vegetables of 0.3 mg kg\(^{-1}\) FW and considering an average DW/FW ratio of 0.2 in ryegrass, the Lolium cultures of many stations of our study would not be approved for human consumption (EU, 2006). This holds especially true for the Spanish cities, which reached maximum site means of up to 14 mg kg\(^{-1}\) DW. Ryegrass is of course not consumed by humans and accumulation capacities of grasses and leafy vegetables will surely differ. But, assuming that accumulation patterns in vegetables might be roughly similar to those of grass cultures, such accumulation in bioindicator plants may be treated as kind of early warning that foodstuffs may also be under risk. In this context, it should also be mentioned that, after the final ban of leaded petrol in Spain in summer 2001, Pb contents in grass cultures fell to levels comparable to those of other European cities (Klumpp et al., 2004).

Zinc, as an essential plant nutrient, is not toxic at the concentrations found in our study. Maximum site means of up to 75 mg kg\(^{-1}\) DW were far below recommended limit values for animal feed (300–500 mg kg\(^{-1}\) DW\(_{12}\), VDI, 1996–2007). Official regulations for feed and food do not currently contain any limit values for Sb and Cr.

5. Conclusions

The present study represents the largest study on trace element pollution in urban centres in Europe that did not focus on assessment of pollution load by determining concentrations and deposition rates of PM and chemical analysis of dust material, but instead focused on the accumulation of trace elements in bioindicator plants. Our study also demonstrated that pollution levels differ considerably between urban/suburban sites and rural/remote sites, as well as between different cities and regions in Europe. Based on our studies, the following conclusions can be drawn:

- The exposure of Italian ryegrass according to the method defined by a technical guideline (VDI, 2003) proved to be a simple and reliable tool for assessing and monitoring the distribution of trace element pollution in urban areas throughout Europe. The highly standardised methodology worked well under varying climatic conditions and provided valuable data on distribution of airborne trace elements within local networks and among different regions in Europe, thus fulfilling the recommendations of the EU Directive (EU, 2004). When comparing data from different local networks, it must be taken into consideration that deposition of pollutants to and leaching from plant tissues is influenced by weather conditions during the exposure period. The same weather conditions, however, also apply to the vegetation growing in situ. The grass culture method thus allows for a time and space integrated analysis of the exposure of plants to air pollution.

- Ryegrass is obviously not part of the human diet, nor does animal husbandry play a major role in city centres. Nevertheless, the use of a fodder plant as a bioindicator of air pollution renders it possible to draw conclusions on the potential entry of contaminants into the food chain and on the potential risk posed to human and animal health. This can be accomplished by comparing biomonitoring data with legally binding limit and threshold values established for animal feed and for foodstuffs.

- The health risk of strong Sb accumulation at heavily-trafficked sites cannot be estimated on the basis of similar regulations and directives as no limit values for this element have been fixed yet. The recent increase of environmental Sb levels due to abrasion from brake linings, however, should be further monitored and investigated because of the element’s known toxicity.

- Data of our investigations represents the pollution situation in a specific study year. The exposure of standardised grass cultures, especially after establishing a widely accepted and adopted European standard, will be a useful tool to monitor and to assess ambient levels of potentially toxic compounds of PM such as heavy metals or certain organic contaminants on a regular basis. Moreover, the method provides the opportunity to observe and document time trends and to demonstrate the increasing or decreasing environmental relevance of certain pollutants.

- In this way, biomonitoring with grass cultures will contribute additional information on environmental and human health aspects to the routine monitoring programmes that deliver data on environmental PM levels from continuous measurements, but do not provide details on the elemental composition of fine dust.
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