Science of the Total Environment 508 (2015) 95–100

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Declining trends of PCDD/Fs in lichens over a decade in a Mediterranean area with multiple pollution sources

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HIGHLIGHTS

- We measured PCDD/F concentrations in lichens over a decade, from 2000 to 2011.
- PCDD/Fs in lichens have decreased approximately 70%.
- This decline couldn't be only explained by the reduction in industrial emissions.

ARTICLE INFO

Article history:
Received 5 August 2014
Received in revised form 20 November 2014
Accepted 20 November 2014
Available online xxxx

Editor: J. P. Bennett

Keywords:
Dioxins and furans
Lichens
Industrial
Urban
Biomonitors
Air quality
POPs

ABSTRACT

Lichens are one of the most useful environmental biomonitors, due to their ability to clearly reflect atmospheric deposition of pollutants. Dioxin and furan (PCDD/F) emissions have been reported to be decreasing in North European countries as a consequence of European regulations. This reduction has been perceptible across several environmental matrices, but it hasn't yet been shown in lichens as typical biomonitors of atmospheric pollution. In this work we compared concentrations of PCDD/Fs in two lichen species collected in a Mediterranean area with mixed land-uses, encompassing urban, industrial and natural areas, in 2009 and 2011 with the ones obtained in the same species collected in the same region in 2000. We found that PCDD/F concentrations in both lichen species have decreased approximately 70% since 2000 whereas industrial emissions have only decreased 25% for the same period. This substantial greater reduction observed in lichens may be due to several causes; after excluding fires as a possible explanation, we point out that possible causes could not only be the overall decrease in industrial emissions but also other causes such as traffic reduction and/or increase efficiency in the use of fuels.

Capsule: PCDD/F concentrations in lichens have decreased 70% over the last decade, whereas industrial emissions have only decreased 25%.

1. Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are toxic environmental pollutants generated during combustion processes, both of natural and anthropogenic origin. They were never intentionally manufactured; rather, they are byproducts of the synthesis and combustion of chlorinated organic chemicals such as chlorophenols and other chlorinated aromatic compounds. Thus, although the production of such chemicals is heavily regulated in most countries, these contaminants are ubiquitous and persistent in the environment (Rappe, 1993; Fiedler, 2003).

Global agreements to reduce PCDD/F emissions and introduction of strict emission limits for European industries during the last decade have reduced emission of PCDD/Fs from industrial facilities (LRTAP, 1979; Stockholm Convention, 2000; Directive 2000/76/EG, 2000; Bruckmann et al., 2013). A review of emission data reported under the LRTAP Convention and NEC Directive of 2012 has stated that PCDD/F emissions in European countries have decreased from 1990 to 2010, showing a declining trend that has been taking place due to regulations

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http://dx.doi.org/10.1016/j.scitotenv.2014.11.065
0048-9697/© 2014 Published by Elsevier B.V.

(Mareckova et al., 2012). Between 2000 and 2011 (the time scale of our study), an approximately 60 percent drop in PCDD/F emissions for the 27 countries of Europe was registered (EEA, 2013); whereas specifically for Portugal, a 25 percent drop in emissions from 2000 to 2011 was observed (PEA, 2013). These reductions were attributable to the introduction of strict emission limits for European waste incineration plants and for industrial installations (Directive 2000/76/EG, 2000) which seem to be effectively reducing industrial emission of PCDD/Fs (Bruckmann et al., 2013).

Most studies related to trends on PCDD/Fs have been carried out in countries where heavy industry was built specifically after World War II. During this period there was an extensive promotion of industrial activities, which have been responsible for wide spread contamination of the environment, including air, soil and biota that might still be reflected in sink compartments such as sediments (Chi et al., 2009; Yunuén et al., 2011). Only after many years technology was improved and adopted by these early industrialized countries, and emissions of pollutants were reduced. Portugal, because of political reasons, experienced a later and less widespread industrialization, and newborn industries have used most recent and improved technology. Because of this later and cleaner industrial development, we expect the country to have less historical accumulation of persistent pollutants in the environment and thus it's predictable that there was a lower drop on PCDD/F emissions than other European countries for the same period.

However, emission from other sources, such as vehicles, and fireplaces, is yet to be quantified and regulated. With the introduction of limits for industrial emissions, these other sources, as well as natural (e.g. forest fires) and probably unknown sources might become more relevant.

Though measures to reduce industrial emission of PCDD/Fs have been implemented, monitoring programs showing the impact of this reduction on the environment at deposition level have seldom been conducted, especially in the decade 2000–2010 and in Mediterranean countries where dry deposition is an important route of pollutant clearance from atmosphere. Most studies covered the time scale of the 1990s or measured a single time PCDD/F deposition in the environment (Alcock and Jones, 1996).

Measuring PCDD/F deposition to the environment directly is difficult; in fact, deposition is commonly estimated from concentrations measured in air, precipitation, and soil (Wagrowski and Hites, 2000). All of these have restrictions; for example, because air is sampled for short periods (usually 24 to 48 h), PCDD/F concentrations tend to be below detection limits of analytical equipment (Gusev et al., 2011). However, since PCDD/Fs are lipophilic compounds with tendency to bioaccumulate, even if not detectable in air, they can be found in biota and soil. Soil is considered a natural sink for persistent organic pollutants; PCDD/Fs absorb to soil organic carbon, and once absorbed, remain relatively immobile (Fiedler, 1999). For this reason, concentrations in soil accumulate the long-term deposition during decades and consequently are not helpful to assess the impact of the recent measures to reduce emissions that have been taking place.

Alternative methods consider PCDD/F concentrations in vegetation as a measure of deposition; in this case, accumulation is dependent on the phenology of the plant (duration of leaves), which may be a disadvantage when the main objective is to obtain temporal trends. Besides, as plants absorb nutrients from soil through roots, they don't represent an independent measure of the present-time atmospheric deposition.

Lichens (symbiosis of fungi and algae or cyanobacteria), on the other hand, have neither roots nor a cuticle to control uptake of elements from the external environment, and thus absorb pollutants directly from atmosphere and accumulate them inside their tissues over time. This characteristic is particularly useful to monitor PCDD/Fs that exist at low concentrations in the environment. Lichens respond quickly to atmospheric changes in pollutant levels, though they still keep a signal of past pollution events (Munzi et al., 2011). Several works showed that lichens can indicate deposition of most atmospheric pollutants, including PCDD/Fs. They've been used to characterize spatial and temporal patterns of a wide range of atmospheric pollutants, ranging from metals to persistent organic pollutants (Augusto, 2012; Schrlau et al., 2011; Usenko et al., 2010; Branquinho et al., 2008; Branquinho, 2001; Manning and Feder, 1980; Martin and Coughtrey, 1982; Puckett, 1988; Garty, 1993). Though a set of studies has been published regarding the use of lichens to monitor PCDD/Fs (Augusto et al., 2013), an insight on temporal trends of these compounds in lichens is still missing.

The main aim of this study was to compare concentrations of PCDD/Fs in two lichen species collected in four sampling periods over a decade (2000–2011) in a Mediterranean area with mixed land-uses, encompassing industrial, urban and natural areas. We hypothesize that PCDD/F concentrations in lichens will show a decrease trend over time that follows the general trend of decrease of Portuguese emissions.

2. Material and methods

2.1. Study area

The study was conducted in an industrial/urban Mediterranean area in Europe (Portugal, Setúbal region) (Fig. 1). This area includes the Serra da Arrábida Natural Park, which covers 108 km^2 and is one of the 30 areas which are officially under protection in the country; and it is surrounded by urban and industrial areas (ICNF, 2013).

Relevant industrial activities comprise cement manufacturing using alternative fuels, production of electricity (using fossil fuel), treatment and disposal of non-hazardous waste, manufacture of fabricated metal products, manufacture of pulp, collection of hazardous waste, manufacture of pesticides and other agrochemical products, repair and maintenance of ships and boats, sea port activities, manufacture of plastics in primary forms, recovery of sorted materials, manufacture of motor vehicles, and manufacture of food and beverages (E-PRTR, 2014).

Urban areas include small villages scattered in the region and the city of Setúbal, summing to 233,516 inhabitants in 2011 (12% more in relation to 2001) (INE, Censos, 2011). Facing partially the Atlantic Ocean and partially the Sado River, the study area also includes recreation and bathing areas and an industrial harbor which serves the cement manufacturing plant. The region is crossed by important motorways and several roads.

2.2. Sampling

Sampling took place in February 2009, October 2009 and March 2011. At each sampling period, 19 to 20 samples of the fruticose lichen *Ramalina canariensis* Steiner and 8 to 11 samples of the foliose lichen *Xanthoria parietina* (L.) Th.Fr. were collected at 29 sampling sites from the available phorophytes — *Pinus pinea*, *Pinus pinaster*, *Olea europaea*, *Quercus suber*, and *Quercus coccifera* (Fig. 1). Previous studies have shown that there are no significant differences in the accumulation of PCDD/Fs by lichens growing on different phorophytes (Augusto et al., 2009). Approximately, 12 g of lichens (whole thallus) was collected at each sampling site.

Due to the variety of land uses existent in the area it wasn't possible to collect the same lichen species over the whole sampling area. Both species that were collected are ubiquitous but occupy sites with different land uses. While *X. parietina* is a very tolerant species found in highly polluted sites, *R. canariensis* is mostly found in forests and natural areas.

After collection, lichens were stored in plastic bags and transported to the laboratory, where the unwashed samples were immediately dried at room temperature (as lichens are poikilohydric, they dry as quickly as 24 h when left in a dry environment) and sorted to remove extraneous material. The cleaned samples were then stored in brown glass bottles until PCDD/F chemical analysis.

In 2000 an environmental biomonitoring baseline study was carried out in the same region as the current study. The same lichen species

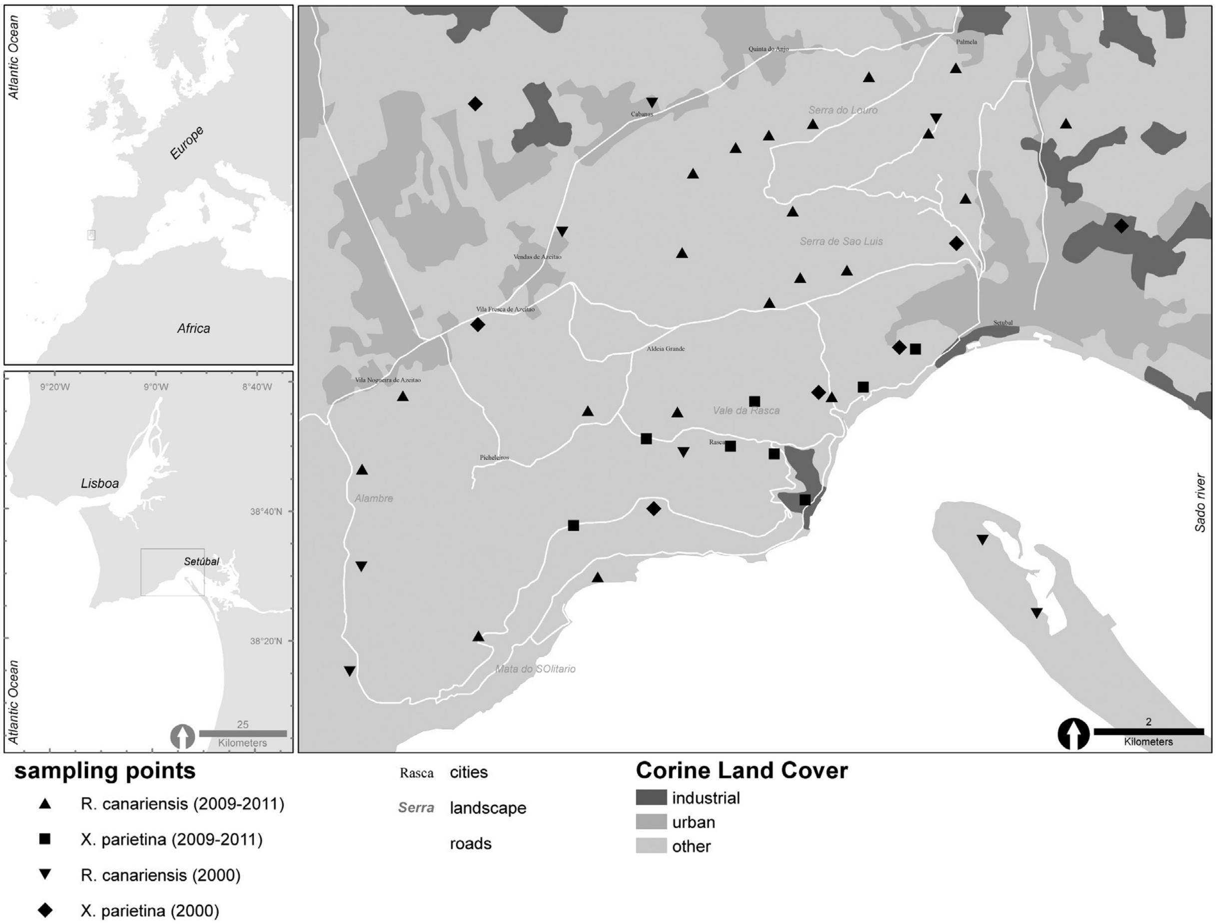

Fig. 1. Study area in the southern part of Portugal, Europe, showing urban, industrial and other land-use classes, and location of lichen sampling sites for 2000, 2009 and 2011.

R. canariensis and *X. parietina* were collected from 110 sampling sites distributed over the region and concentrations of PCDD/Fs were quantified (Life98 ENV/P/000556; Augusto et al., 2004, 2007a,b). The sampling procedure was similar to the one adopted in the current study; details are reported elsewhere (Augusto et al., 2004, 2007a,b).

2.3. Analytical procedure

PCDD/F analyses in 2009–2011 took place at the certified laboratory Eurofins GfA Lab Service GmbH, in Germany, which is accredited for determining dioxins (PCDDs) and furans (PCDFs) in plant matrices in accordance with DIN EN ISO/IEC 17025:2005. PCDD/F analysis in lichens followed the Internal Standard GLS DF 100, HRMS. The analytical steps are as follows: sample preparation (homogenization), addition of all PCDD/F components to be determined as internal $^{13}C_{12}$ marked PCDD/F standard substances, extraction in Soxhlet, clean-up of the extract by way of column chromatography, analysis by means of high resolution mass spectrometry (HRGC/HRMS), and quantification of the native PCDD/Fs via the internal $^{13}C_{12}$ marked standards (isotope dilution method).

PCDD/F analyses in 2000 took place in the specialized analytic laboratory TERRA PROTECTA in Berlin, Germany, which has a German Accreditation for Dioxin Measurements. This laboratory has experience with mosses and vegetables, being also a partner lab in a European monitoring project for mosses (*Brachythecium rutabulum*) for analyzing PCDD/Fs. The PCDD/F analysis was executed following the US EPA 1613 B protocols. Ground and dried lichen samples were extracted using the Soxhlet method (toluene). Cleanup was based on a mixed silica column, aluminum oxide column and gel-chromatography (Bio-Beads S-X3A) method. Measurement of dioxins was performed using gaseous chromatography and high-resolution mass spectrometry (HRGC/HRMS) using a Varian 3400 gas chromatograph equipped with a cold injection system (Gerstel KAS) and a DB-Dioxin column, and a Finnigan MAT 90 HRMS at a resolution 8000–10,000. Using the isotope dilution method, $^{13}C_{12}$-labeled internal standards were added prior to the extraction; the surrogate for determination of the recovery ratio was added just prior to analysis. Recovery ratios of the labeled internal standard added to each sample prior to the extraction were in the range 60–120% for all samples analyzed. The method used is accredited according to the ISO 17025 standard (EN 45001, 2002) covering all required QA/QC measures such as, blank controls, certified reference materials, and inter-laboratory comparisons.

For all samples, the seventeen toxic PCDD/Fs (the ones with at least 4 chlorine atoms in positions 2, 3, 7 and 8 of the molecule) were quantified. Results were further displayed as the sum of concentrations of the 17 toxic PCDD/Fs ($\sum$17 PCDD/Fs).

For samples in which a compound was not detected (ND), its concentration was assumed to be the detection limit value. Due to the improvement of analytical methods from 2000 to 2009–2011, it would be expected that assuming that detection limit values for under detected compounds would contribute to obtain higher concentrations in samples from 2000 than in the current ones.

However, detection limits in 2000 were in the same range as the current ones for most of the compounds, and even lower for some compounds. From the 272 analyses performed in 2000 (analysis of 17 congeners in 16 lichen samples), 6 yielded values below detection limits, which correspond to 2.2% of the analyses (LOD varied between

0.1 and 0.3 ng kg^{-1} depending on the compound). From the 1309 analyses performed in 2009–2011 (analysis of 17 congeners in 77 lichen samples), 156 yielded values below detection limits, which means 11.9% of non-detectable values (LOD varied between 0.1 and 23 ng kg^{-1}, depending on the compound).

2.4. Temporal analysis

Because sampling points did not coincide between dates (Fig. 1) an interpolation of the values for one of the dates was necessary. The 2000 sampling was chosen due to the higher number of sampling points available, which results in more robust interpolation models.

For the interpolation an ordinary kriging was chosen. This geostatistical method is a robust interpolation procedure commonly used in pollution studies. For that, the 2000 values of PCDD/Fs of *R. canariensis* ($n = 44$) and *X. parietina* ($n = 66$) were analyzed by variography. For the *X. parietina* values the highest values were excluded from the variogram analysis (but were later included in the interpolation). Both variograms (twelve 2500 m lags) revealed a strong spatial structure, and a reduced nugget effect, assuring the robustness of the interpolation. The two variables were then modeled with a circular isotropic function with zero nugget effect and a range of 9500 m for *R. canariensis* and 7000 m for *X. parietina*. Using these functions the variables were interpolated for the study region. The values of each variable in the location of the 2009–2011 sampling were then retrieved and used for further analysis.

For each lichen species, descriptive statistics (mean, standard deviation, median, minimum and maximum) were used to characterize PCDD/F concentrations along the four sampling periods (from 2000 to 2011) (Table 1). Box plots with the median values were plotted for visual interpretation (Fig. 2). The nonparametric Mann–Whitney *U* test was conducted to test for significant differences between PCDD/F concentrations in both lichen species in 2000 and 2011. Statistical analysis of the results was carried out using STATISTICA 10.0 StatSoft Inc. Package.

3. Results and discussion

Table 1 summarizes PCDD/F concentrations found in the lichen species *R. canariensis* and *X. parietina* during the four sampling periods of this study (March 2000, February 2009, October 2009 and March 2011). Concentrations of PCDD/Fs decreased significantly for both species over time from 2000 to 2011 (68.20 per cent drop for *R. canariensis* and 72.71 per cent drop for *X. parietina*) (Table 1 and Fig. 2) (Mann–Whitney *U* test, $p < 0.0001$ for both species). Yearly loss rates since 2000 of 9.36 ng kg^{-1} (corresponding to 6.20%) and 7.38 ng kg^{-1} (corresponding to 6.61%) were found for PCDD/F mean concentrations for *R. canariensis* and *X. parietina*, respectively (Table 1). By displaying the box-plots, it's possible to visualize the range of values associated with each sampling period (Fig. 2).

Table 1
Summary of PCDD/F concentrations (ng kg^{-1}) found in the lichen species *R. canariensis* and *X. parietina* collected in the four sampling periods of this study: March 2000, February 2009, October 2009 and March 2011. Percentage of loss between 2000 and 2011 and yearly loss rates are also displayed.

		March 2000	February 2009	October 2009	April 2011	% loss (2000–2011)
R. canariensis	Mean	151.75	85.11	77.50	48.78	68.20
n = 18	SD	22.17	36.02	37.85	21.58	12.29
	Med	160.03	79.00	72.84	51.08	Yearly loss rate
	Min	106.52	26.30	37.51	12.37	(ng kg^{-1})
	Max	175.70	162.14	197.33	85.58	9.36
X. parientina	Mean	111.71	52.99	49.82	35.18	72.71
n = 8	SD	14.59	17.57	8.75	17.66	9.11
	Med	114.68	49.35	49.71	32.00	Yearly loss rate
	Min	87.07	36.61	35.90	19.23	(ng kg^{-1})
	Max	130.59	93.43	59.76	74.56	7.38

PCDD/F concentrations in lichens decreased from 2000 to 2011, following a declining trend that has been also reported for emissions. However, the 70 percent drop observed in lichens was larger than the one reported for Portuguese emissions (only 25% drop) not in accordance with the posed hypothesis. We suggest that this larger decrease observed in lichens might be due to either the decrease of other sources of PCDD/Fs which are not reported in the emission inventory (such as forest fires) or to the larger decrease of emissions in this particular region of Portugal compared to the national average.

In areas with Mediterranean climate, during the driest seasons, forest fires may be considered a major emission source of several pollutants, including particles, PCDD/Fs, and polycyclic aromatic hydrocarbons (PAHs). Because most studies reporting PCDD/F environmental monitoring have been carried out in Northern countries where forest fires are of less importance, this source has been less studied.

One of the possible explanations for the 70 percent drop in PCDD/F concentrations in lichens could be variations in fire emissions. The most recent estimation of PCDD/F atmospheric emissions in Portugal covering the period 2004–2009 and which included 27 emission source types, showed that only 8 source types were relevant, with forest fires being the largest and most variable one, followed by wood preservation, residential wood combustion, sintering and steel production on electric arc furnaces (Relvas et al., 2013). They also suggested that as industrial sources have been curtailed, natural sources such as forest fires have overtaken other sources in significance.

Our study region includes a Natural Park which has been subjected to several forest fires over time; the average value of the burnt area per year from 1992 to 2000 in our study region was 172 ± 189 ha whereas the median value was 93 ha. From 2001 to 2010 the average value of the burnt area per year increased to 680 ± 879 ha and the median value to 350 ha (ICNF, 2014). Burnt area has therefore increased from the decade 1992–2000 to 2001–2010, thus in this area forest fires couldn't explain the regional declining trends of PCDD/F concentrations in lichens.

Urban areas with high population densities are usually considered diffuse sources of PCDD/Fs, with traffic being one of the responsible sources (Kuykendall et al., 2009). Quantifying the amount of anthropogenic activities in urban areas is a challenging task that needs to be addressed indirectly through analysis of population indicators. The number of inhabitants in the study area has increased 12% from 2001 to 2011 (INE, Censos, 2011). Consumption of electric energy has also increased by 25% from 2000 to 2011 (22% from 2001 to 2011) (INE, 2014). These two indicators show a growing trend of anthropogenic activities, which would be expected to contribute to an increase of PCDD/F emissions rather than to a decline. However, data for fuel sales in the region (there was no data available for our restricted study area), including data for petrol and diesel, show a decrease of 13% from 2001 to 2011, which could be interpreted as a reduction of traffic flow and/or emissions (Pordata, 2014). This interpretation is supported by a drop in concentrations of PM_{10} (particles < 10 μm) (≈25%) and nitrogen oxides (nitrogen monoxide and nitrogen dioxide) (≈40 and ≈50%, respectively) from 2002 to 2011 measured at an air quality monitoring station located in the main city of the study area in comparison with a background station (Qualar, 2014). Moreover, combustion in vehicles has become itself more efficient over time and thus pollutants deriving from traffic have been decreasing (Bernard et al., 2001; Kuykendall et al., 2009). However some studies suggest that traffic account only for approximately 3 to 9% of PCDD/F emissions from known sources (Defra, 2002; PEA, 2013).

In resume, the 70 percent drop in PCDD/F concentrations in lichens from 2000 to 2011 cannot only be explained by the reduction in Portuguese industrial emissions (25% drop) but most probably is also related to the reduction in vehicle emissions both due to a reduction on traffic intensity itself and to an increase in petrol use efficiency. These results

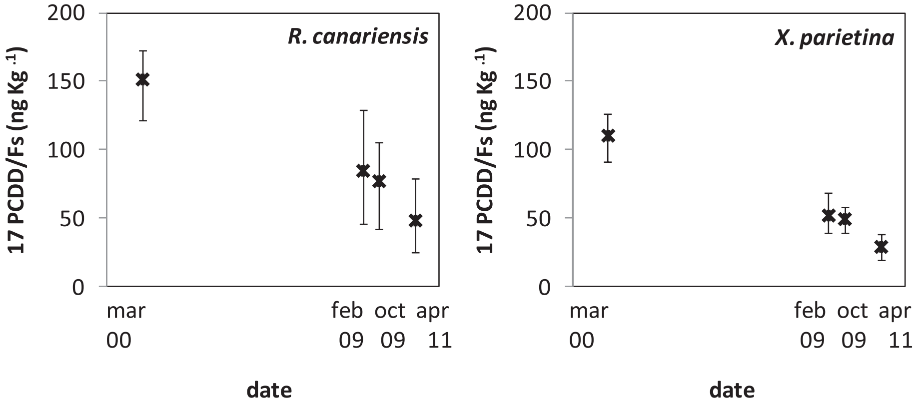

Fig. 2. Temporal variation of concentrations of the $\sum 17$ PCDD/Fs measured in two lichen species, *R. canariensis* and *X. parietina*, between 2000 and 2011. Crosses represent the median values, lines the 10% and 90% percentiles. Sampling dates were: March 2000, February 2009, October 2009 and March 2011.

highlight the need to further understand the relative importance of vehicle emissions in urban areas. It also highlights the importance of using environmental biomonitors of pollution that are able to integrate all sources of pollution and track its dynamics over time.

Processes by which lichens accumulate PCDD/Fs are yet to be studied. However, for most pollutants (especially metals), accumulation occurs after dry or wet deposition through a set of different mechanisms, notably: adsorption of particles to lichen's surface, incorporation into cell walls through ion exchange processes, and metabolically controlled passage into the cells (Branquinho et al., 1999; Branquinho, 2001; Tyler, 1989). It's logical to expect that similar processes are responsible for accumulation of PCDD/Fs if they are in the particulate phase. The most likely is that these compounds will bind to lipids both on lichen's surface and inside the thallus. In the case of particles retained to lichen's surface, these may be washed under strong wet events whereas others can be intercepted by the fungi hyphae and remain there for years, giving a memory effect (for a review on the methods of particles uptake see Branquinho, 2001). Those accumulated will probably be diluted with lichen growth and/or fragmentation. However, this is a matter that needs further investigation, as PCDD/Fs are chemically different from pollutants whose accumulation by lichens have been studied so far.

4. Conclusions

This work represents the first analysis of temporal trends in PCDD/F concentrations in lichens over a decade published so far. Our data showed that PCDD/Fs in lichens decrease by 70% whereas industrial emissions only reduced 25%. The difference might be associated with the reduction in vehicle emissions highlighting the need for further research on this area.

Acknowledgments

The authors are grateful for the financial support from Secil Companhia Geral de Cal e Cimento S.A., Life Environment program ENV/P/000830 and FCT-MEC (Investigador FCT and SFRH/BPD/75425/2010). Sofia Augusto acknowledges the European Union Seventh Framework Programme for the Marie Curie Intra-European Fellowship for Career Development (FP7-PEOPLE-2013-IEF-624022).

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