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The contribution of environmental biomonitoring with lichens to assess human exposure to dioxins

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Abstract

The contribution of environmental biomonitoring with lichens to assess human exposure to dioxins was the main purpose of this work. For that, polychlorinated dibenzofurans (PCDD/F) were measured in 66 lichen sampling points. The obtained information significantly improved the basic knowledge on the environmental exposure to dioxins through distinction between effective control areas from areas with moderate atmospheric deposition. It allowed the integration of PCDD/F atmospheric deposition for much longer periods, allowing to relate low levels with long-term chronic effects on health. Thus, the production of high-resolution data on environmental exposure essential to perform reliable environmental health studies was possible. It was argued that PCDD/F in lichens may be used as spatial estimators of the potential risk of inhalation by the population present in the area. An example of the application of this data to select control and exposed areas for environmental health studies was presented.

Keywords: Lichens; Inhalation; Environmental exposure; PCDD/F; Geostatistics; Environmental health; Biomonitoring

Introduction

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/F) are organic compounds formed as unwanted by-products of combustion in many industrial chemical processes, and have been detected in almost all environmental matrices such as soil, sediment, air, water, animals and vegetation (Rappe, 1993; WHO, 1992; Fiedler et al., 1990). These compounds are very important in environmental health studies because they are carcinogenic and potentially toxic, even in very low concentrations. Atmosphere is a major pathway for transport and deposition of PCDD/F on different environmental media.

Human health risk assessment requires identification of the pathways through which people can be potentially exposed to these chemicals (Meneses et al., 2004). The quantitative estimation of health risk due to an environmental exposure can be considered as a combination of five pathways, namely air inhalation, foodstuffs (including breast feeding), drinking water, absorption through skin and soil ingestion (Meneses et al., 2004). Although the main route of PCDD/F human contamination is through food ingestion, for an accurate health risk estimate it is essential to evaluate every other exposure route; hence, the pollutant

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concentrations in each environmental medium must be determined (Nessel et al., 1991).

In epidemiological studies, the environmental observations must be made in a way that reflects as closely as possible the exposure of the population under observation. Since such a level of information is difficult to attain, many studies are based on estimated exposure from data obtained at monitoring sites previously selected for regulatory purposes rather than for estimating the real exposure of the population (EHC 27, 1983). Sampling sites tend to be selected for their expected relatively high concentrations and are often placed at a much higher altitude than the human breathing zone (EHC 27, 1983).

The most acceptable way to perform an accurate measurement of the environmental level of a particular pollutant is through a biomonitoring program (EHC 27, 1983). Environmental biomonitoring consists in the monitoring of the pollutant levels in the environment by means of living organisms (Martin and Coughtrey, 1982; Puckett, 1988; Sloof, 1993). Lichens, the most studied biomonitors of air pollution, are symbiotic organisms consisting of fungi and algae or cyanobacteria. Many authors have pointed out that lichens have several of the characteristics required for the ideal biological monitor (Manning and Feder, 1980; Martin and Coughtrey, 1982; Nimis, 1990; Garty, 1993; Sloof, 1993; Branquinho, 1997, 2001). They have been used because they are sensitive to variations in environmental conditions with a change in their "signs of activity" (Markert et al., 1997). These changes may occur (i) in the structure or dynamics of the population or community of organisms, (ii) in changes in their functional response, which may lead to changes in vitality, and (iii) in concentrations of elements which influence the organism (Branquinho, 2001).

Lichens have been used to biomonitor several pollutant levels, particularly of sulphur, nitrogen, fluoride, oxygen, metals, radionuclide, dioxins, other organic compounds, etc. (Martin and Coughtrey, 1982; Puckett, 1988; Garty, 1993; Augusto et al., 2004a, b, c). The concentrations of given pollutants, measured within the organism, are used to reconstruct the spatial and temporal deposition patterns of the pollutants deposited at a location. As a result, lichens have been very helpful in identifying several anthropogenic sources such as roads, mines and industrial facilities in urban and rural locations (Branquinho, 2001), and natural sources such as volcanoes.

Recent work on the performance of lichens as biomonitors of organic compounds, particularly PCDD/F, has shown the potential of these organisms for monitoring PCDD/F atmospheric deposition (Augusto et al., 2004a, b, c). The goal of this study is to show how environmental data obtained through biomonitoring with lichens can be integrated in environmental health studies.

Materials and methods

Lichen sampling

The area studied was Setúbal peninsula, located in south Portugal, covering an area of 150,000 ha. The lichen species selected for biomonitorization of PCDD/F atmospheric deposition was *Xanthoria parietina* (L.) Th. Fr. growing on house roof tiles (Augusto et al., 2004a). Lichen sampling was performed during March 2000, after a dry period of 84 days (precipitation below 7 mm), during a meteorologically stable period, at 66 locations.

PCDD/F analysis

After collection, the lichens were stored in plastic bags and transported to the laboratory, where the unwashed samples were immediately dried at room temperature and sorted to remove extraneous material (other lichen or moss species). The cleaned samples (ca 15 g) were then ground (Glen Creston Ltd. MM 2000) and analysed for PCDD/F concentration. Ground and dried lichen samples were added with labelled standards $^{13}C_{12}$, subjected to toluene extraction and purified to remove intrusive substances. PCDD/F were quantified by gaseous chromatography and high-resolution mass spectrometry (Fisons Autospec Ultima System). The precision and accuracy of the analysis was checked against reference material. Toxicity of each sample was determined through the sum of the concentrations (ng I-TEQ kg^{-1}) calculated for the toxic congeners.

Demographic indicators

The demographic indicators "resident population" and "resident population under 14 years old", published by National authority in statistics (INE, 2006), at the parish level were used for the following investigation.

Mapping environmental and demographic indicators

The PCDD/F concentrations measured in lichens samples were assumed as indicators of PCDD/F atmospheric deposition levels. This data set shows a positively skewed distribution. The experimental semivariograms were calculated and, as they did not show any preferential direction of spatial dispersion, an isotropic semivariogram model comprising two spherical structures was fitted with ranges of 6 and 26 km. Based on the fitted semi variogram model, a map of PCDD/F atmospheric deposition levels was estimated using ordinary kriging (geoestatistical estimator) on a regular grid of 310×200 nodes with a grid spacing of 200 m. To perform all the geostatistical calculations the program geoMS – Geostatistical Modelling Software (CMRP-IST, 2000) was used. Then, using ArcGIS Desktop 9.1 (ESRI INC, 1999–2005) a geographical information system was built gathering all data together, namely, parish limits, demographic data and environmental data, in the same geographical referential. Afterwards, using geographical information systems tools, PCDD/F atmospheric deposition levels statistics at a parish level were calculated, such as means and standard deviations, obtaining a set of maps to which demographic indicators were overlapped, for helping in interpretation and results' discussion.

Results and discussion

Environmental assessment of exposure to dioxins

In order to assess environmental exposure to dioxins, data obtained through the chemical analysis of lichens collected in the region under study were processed by means of geostatistical methodologies. Thus, the production of high-resolution maps with important information regarding the deposition of these compounds in the region significantly improved the basic knowledge on the environmental exposure, which is critical for an adequate study of the impact of PCDD/F on human health.

Environmental health studies devise several questions: which populations are (or have been) exposed to the pollutants; which populations should be selected for further human biomonitoring studies; and which should be considered as control populations? To answer those questions it is necessary, firstly, to develop a monitoring program on the environmental levels of the pollutants under study (EHC 27, 1983). In air quality monitoring programs some details should be kept in mind: pollutants discharged to the atmosphere are not constant in space and time. Usually, air quality data are either sparse in space or reflect short periods of time (in the order of seconds or minutes). Furthermore, in the case of PCDD/F there are no monitoring stations continuously measuring the levels of these pollutants.

When compared to other environmental biomonitors or monitoring stations, lichens integrate much longer periods of pollutants' atmospheric deposition (from months up to 5 years) (Coutinho et al., 1999; Branquinho, 2001; Simonetti et al., 2000). This kind of data is more appropriate for comparisons with health data than the short-term measurements normally performed in the air or even in plants, which report from few hours to days or to a season, respectively. In this way, lichens provide a sample of the complex mixture of PCDD/F that humans and biota have been exposed to in a long term. This will be of critical importance for health studies, since one of the most difficult tasks is to relate the low pollution levels with long-term chronic effects on health (Mukerjee and Cleverly, 1987). Moreover, lichens act as simple biological models for pollutants' deposition and effects, both on humans and ecosystems. For the case of health in humans, this was shown by the work of Cislaghi and Nimis (1997), where lichens diversity used as bioindicator of atmospheric pollution showed a good correlation with lung cancer in north-east Italy.

Concerning the spatial coverage of physical monitoring networks, a single site may be assumed to represent a large area and the number of sites is often limited because of operative and financial constraints related to the installation of a monitoring station. Lichens allow the adoption of cost-effective sampling strategies with relatively high density of sampling locations, thus generating more spatially detailed data in order to obtain high-resolution maps. They have a wide geographical distribution allowing comparison of pollutant concentrations from diverse regions. The maps produced in this work represent the only study of PCDD/F deposition in Portugal with high spatial resolution and consequently a reliable spatial model. These results have shown that the spatial continuity for PCDD/F deposition has a two-structure semivariogram with 6 and 26 km ranges and that the deposition of PCDD/F occurs both in urban and industrial areas (Augusto et al., 2004a).

On the other hand, levels of PCDD/F in the air measurements are frequently below detection limit, especially in background areas. In this work, all lichen samples were analysed and detectable PCDD/F levels were measured, even in those samples located at the less contaminated areas (ranging from 0.87 to 22.58 ng I-TEQ kg^{-1}). In fact, lichens usually present the highest accumulation of pollutants from the environment in comparison with other living organisms because they are slow-growing, long-lived organisms. This ability of lichens to accumulate PCDD/F is an important advantage of using lichens as environmental biomonitors for human monitoring purposes (Augusto et al., 2004a, b, c). Since these pollutants may affect health even in very low concentrations and bioaccumulate in humans (Boening, 1998; Sweetman et al., 2000; Kogevinas, 2001; Birnbaum and Cummings, 2002), this ability to discriminate very small local spatial variability in atmospheric deposition of PCDD/F may be determinant in distinguishing effective control areas from areas with moderate PCDD/F levels (Fig. 1). In the map of Fig. 1 small neighbour parishes of the same municipality have shown that the levels of atmospheric PCDD/F may differ significantly between them. This implies that populations living there are exposed to contrasting PCDD/F environments through atmospheric pollution, consequently only a high-resolution environment exposure data set is acceptable to perform reliable environmental health studies.

Mapping the PCDD/F inhalation risk estimated by biomonitors

In general, food intake is usually considered the primary source of exposure to PCDD/F, and inhalation and dermal contact are only considered minor routes (Karademir, 2004; Meneses et al., 2004). The level of PCDD/F in food is not a variable related to the local environmental exposure in urban areas, since the populations, in general, tend to consume food from other places. In a prevention context, local decision makers find it difficult to control and regulate potential sources of contamination in non-local foodstuffs. Thus, the impact of local or regional PCDD/F pollution in air and its deposition on the different media has direct and indirect effects on local human health, which should not be neglected. This pathway includes PCDD/F inhalation, dermal contact, soil and dust ingestion, and local food intake. In fact, results of daily intake of PCDD/F only by inhaling air suggested that the inhalation exposure of PCDD/F by the inhabitants in Liwan district, China, is relatively high (Yu et al., 2006). Additionally, prevention measures to reduce the human exposure due to local or regional pollution is much easier to implement than to control and reduce contamination from general food intake.

In several studies, inhalation exposure is calculated by assuming that individuals were exposed to polluted air 24 h day^{-1} and that indoor air exposure was equal to outdoor exposure (Schuhmacher and Domingo, 2006; Yu et al., 2006). These measures directly reflect the concentration and profile of PCDD/F in air and are only weighted by the different ventilation rate of adults and children (Schuhmacher and Domingo, 2006; Yu et al., 2006). Pollutants (heavy metals, radionuclides, etc.) in lichens have been shown to reflect atmospheric pollution (Garty, 2001). Calibrations between the concentrations of pollutants in lichens and those of monitoring stations have been achieved for a series of pollutants (Branquinho et al., 1998, 2004). In the same way, the PCDD/F congener and homologue profiles in lichens were compared to that of the air and soil, and it was found that PCDD/F in lichens were reflecting the air profile (Augusto et al., 2004a). However, calibration is not yet possible due to the lack of PCDD/F monitoring stations.

Lichens are poikilohydric organisms, lacking root systems and a developed cuticle, with very limited control of the uptake and loss of water and solutes from atmospheric deposition. Consequently, they developed other strategies to intercept nutrients from the air, becoming very efficient at it, and reflecting in this way the atmospheric composition. Given the reasons mentioned before, in this work PCDD/F in lichens were considered as spatial estimators of the potential risk of inhalation by the population residing in the same area (Figs. 1 and 3). Further studies, both human biomonitoring and/or epidemiological, may now be performed in order to evaluate the impact of the levels of inhaled dioxins on the total level of PCDD/F in the population. For that, control and exposed areas must be selected. Information such as the one presented in Figs. 1 and 2 may assist this decision. Fig. 1 gives information about the mean concentration of PCDD/F in TEQ's per parish overlaying the number of the resident population in the same territory unit, whereas Fig. 2 gives information on the level of variance associated with each territory unit. Since the lichens sampling in space was regularly distributed, lower standard deviation for a parish reflects consistency between the concentrations found at the sampling locations within the parish, showing that the risk of exposure via inhalation is similar all over the same unit (Fig. 2). In the same way, higher standard deviations show high variability of exposure via inhalation at the same territory unit. A higher number of resident populations is also required to perform representative human health studies. Moreover, in the

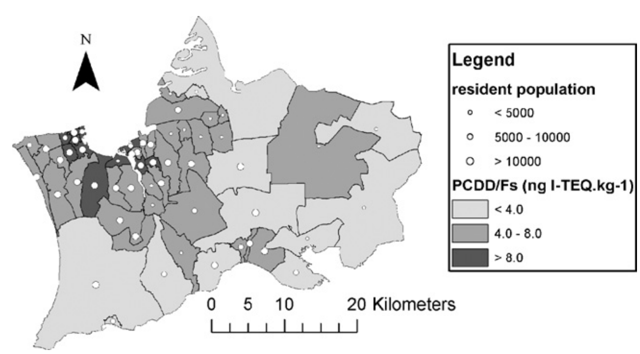

Fig. 1. Mean concentration of PCDD/F in TEQ's per parish overlaying the number of the resident population in the same territory unit.

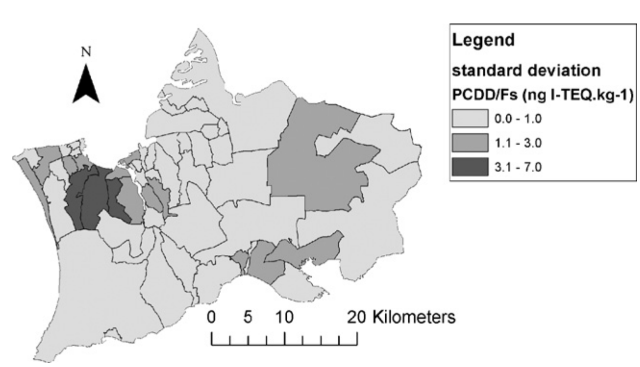

Fig. 2. Level of variance in PCDD/F concentration associated with each territory unit.

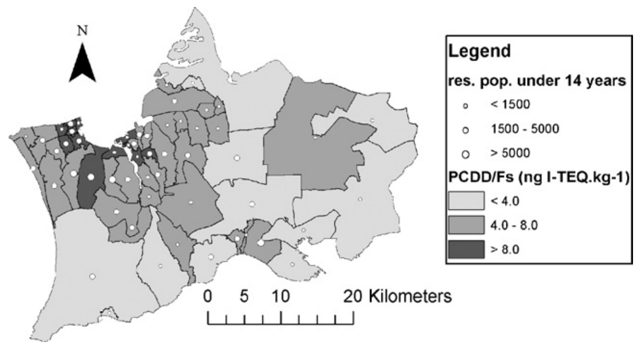

Fig. 3. Level of PCDD/F in lichens overlayed to the infant population (0–14 years).

decision-making process concerning public health, it is advantageous to focus there where the greatest numbers of exposed persons are. In this way, priority should be given to the most contaminated areas where there is simultaneously a greater number of residents.

The number of residents under 14 years can be considered as a risk group regarding PCDD/F, since they are more vulnerable. Children are closer to the soil and are thus more exposed to ingestion and inhalation of soil particles. Although most of the information available on potential age-related differences in the toxicity of PCDD/F comes from experiments in laboratory animals, a number of epidemiological studies evaluated the effects of children's exposure to these compounds. Children accidentally exposed to high levels of PCDD/F either before or after birth have been reported to present a number of developmental deficits (Charnley and Kimbrough, 2006). For that reason, in this work the level of PCDD/F in lichens overlayed to the infant population (0–14 years) was also plotted (Fig. 3). Overall, this environmental information may allow the assisted selection of populations with different potential risks of PCDD/F exposure for further health studies.

Conclusions

The construction of reliable atmospheric pollutant spatial models provides the location of the areas with greatest/lowest pollutant deposition, integrated over time. Those models would be very useful in human biomonitoring and/or epidemiological studies for selecting control and exposed groups of the population. It would allow saving resources because it gives the possibility to focus human biomonitoring and/or epidemiological studies on areas with effective pollution impact.

The regional maps developed in this study can be used to (i) identify critical PCDD/F deposition areas, (ii) optimize PCDD/F monitoring networks and (iii) produce risk assessment studies, including epidemiological investigations. The methodology followed to perform this study can be applied to other regions of the world, thereby contributing to a better knowledge of PCDD/F deposition levels in ecosystems and its impact on human health.

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