PCDD/Fs IN BIOMONITORS: COMPARISON BETWEEN TWO LICHEN SPECIES

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Introduction

Lichens are long-lived extremely sensitive symbiotic organisms consisting of fungi and algae, which are the most studied biomonitors of air pollution. Lichens have been used to biomonitor several pollutant levels, particularly of sulphur, nitrogen, fluoride, oxygen, metals, radionuclide and more recently dioxins and other organic compounds[1-8].

Recent work on the performance of lichens as biomonitors of organic compounds have shown the potential of these organisms for monitoring PCDD/F atmospheric deposition[5-8]. Two lichen species have been used in the last years as PCDD/F biomonitors: *Xanthoria parietina* (L.) Th. Fr., a dorsiventral lichen, leaf-like with well-defined upper and lower surfaces and broadly attached to its substrate (foliose lichen); and *Ramalina canariensis* Steiner, a densely branched and three-dimensional lichen with a single-point of attachment (fruticose lichen). Although these species have been successfully used as PCDD/Fs biomonitors, there are some aspects that need to be studied, such as the difference between species, in order to optimize their use for monitoring purposes.

Understanding their performance for accumulation of PCDD/Fs will be useful to compare monitoring data obtained using these different lichen species and for applications of this work in other regional areas where it cannot be found only one lichen species.

Materials and Methods

The lichen species selected to perform this study were *Ramalina canariensis* and *Xanthoria parietina*. These species were selected because to date they were the single lichens used for PCDD/F biomonitoring[5-8]. *Ramalina canariensis* is easily collected from branches of trees, where it is attached by a single point of fixation, allowing the whole lichen to be exposed to air pollutants. *Xanthoria parietina* is a very tolerant species that can be found growing in much polluted areas, such as in urban and industrial areas. For that, this species is one of the most used in biomonitoring studies.

In order to compare PCDD/F levels and profiles in these two species, lichens were collected at eight sites in a selected region of Portugal, Setúbal pensinsula, which is an important urban and industrial area of the country. In each of these sampling sites, the lichen *R. canariensis* was collected from *Pinus pinea* Aiton, on a minimum of five to ten trees at each sampling point, and always at 1-3 m height, and the lichen *X. parietina* was collected from house roof-tiles. It was also collected a sample of *X. parietina* from house roof-tiles and from branches of *Olea europaea* L. at a ninth sampling site, in order to evaluate the influence of collecting lichens from house roof-tiles or from a phorophyte.

After collection, all samples were store in plastic bags and transported to the laboratory, where unwashed samples were immediately dried at room temperature and sorted to remove extraneous material. The cleaned samples (*c.* 15 g) were then ground, kept in closed glass containers and analysed for PCDD/Fs. The glass containers were kept at room temperature, between 20 and 25 ºC. The PCDD/F analysis was executed following the EPA 1613 B protocol and took place in the specialized analytic laboratory TERRA PROTECTA in Berlin, Germany, which has a German Accrediation for Dioxin Measurements. For metal analysis, ground lichen samples of approximately 100 mg dry weight (lichens dried at 50 ◦C for 24 h in a hot air oven) were digested with 3 ml of nitric acid (65%) at 120 ◦C. Glass tubes with 3 ml of nitric acid and without lichens were used as controls. Zinc, Fe, Mg, Mn, Ca and K were analyzed by atomic absorption spectrophotometry (SpectrAA/50 Varian), using an air/acetylene mixture flame. Before Ca and K analysis, CsCl and LaCl3 (1g/l) were added to the samples to prevent ionization and the formation of refractory compounds. Lead, Cr, Co and Cu were analyzed by atomic absorption spectrophotometry (GBC 932 plus) using a graphite furnace (GBC GF 3000).

Results and Discussion

The concentrations of PCDD/Fs in lichens collected at the same sites ranged between 170.8 and 344.7 ng/Kg in *X. parietina* and from 391.9 to 1058.6 ng/Kg in *R. canariensis* (Table 1). The fruticose lichen *R. canariensis* seemed to accumulate higher concentrations of PCDD/Fs when compared to the foliose lichen *X. parietina*. On the other hand, *X. parietina* showed higher concentrations for all metals analysed except for calcium (Table 2).

Table 1. Statistical summary of PCDD/Fs concentrations (ng/Kg) in lichens collected at the same sites and at the same time. N=8.

	Mean	**SD**	**Min**	**Max**
X. parietina	246.3	65.5	170.8	344.7
R. canariensis	799.7	231.5	391.9	1058.6

Table 2. Statistical summary of metal concentrations (mg/Kg) in lichens collected at the same sites and at the same time. N=8.

mg/Kg	***X. parietina***		***R. canariensis***	
	Mean	**SD**	**Mean**	**SD**
Zn	69.5	21.5	42.1	19.3
Fe	2051.4	964.3	518.8	221.1
Mg	1095.6	324.0	574.1	175.0
Mn	54.3	48.5	21.6	4.9
Ca	1364.8	614.9	5109.9	4089.1
K	3200.4	1938.3	1775.0	1104.5
Cu	23.4	15.7	13.6	4.7
Pb	4.0	2.5	3.0	2.5
Co	13.3	12.4	11.5	10.9
Cr	67.9	87.7	8.5	3.9

Lichen morphology influences the rate at which lichens accumulate elements from the atmosphere[9]. Growth form dictates thallus orientation and the amount of continuous surface area exposed to airborne deposition; therefore, it should have a direct impact on the interception of airborne elements by lichens. *Ramalina canariensis* is bushy-like structured lichen that has a higher ratio surface area/volume than *X. parietina*. This characteristic might facilitate the interception of aerosols and low molecular weight particles by *R. canariensis*. Another possible explanation for the higher values of PCDD/Fs in *R. canariensis* might be related to specific characteristics of the lichen surface that contribute for the retention of lipophilic compounds.

Comparing the homologue profiles of the two species, it can be observed that in *X. parietina* the profile is dominated by the more chlorinated PCDD/Fs, such as OCDD, OCDF, HpCDD and HpCDF, whereas in *R. canariensis* the profile is dominated by the less chlorinated PCDD/Fs (Figure 1a). These differences are not likely to be due to the fact that lichens were collected from different substrates – house roof-tiles and pine – as results from samples of *X. parietina* collected from roof-tiles and from *Olea europaea* showed no differences between homologue profiles (Figure 1b). Moreover, lichens collected from roof-tiles showed higher concentrations of PCDD/Fs than lichens collected at the same site from *O. europaea* (755.9 and 442.1 ng/Kg, respectively).

Results of correlations between metal content and percentage contribution of each homologue to the total PCDD/Fs in lichens are displayed in Table 3. As it can be observed, in *X. parietina* the contribution of the most chlorinated

PCDD/Fs are positively related to metals such as Zn, Fe, Mn, Co and Cr; and the contribution of the less chlorinated PCDD/Fs are negatively related to metals. These results clearly show that *X. parietina* and *R. canariensis* have different types of interception and accumulation of PCDD/Fs. Whereas *X. parietina* mainly reflect the most chlorinated PCDD/Fs, the lichen *R. canariensis* mainly reflect the less chlorinated PCDD/Fs. Some authors argue that most chlorinated PCDD/Fs are more stable in the environment than the less chlorinated PCDD/Fs[10,11]. The higher contribution of the most chlorinated PCDD/Fs in *X. parietina* can be related to the higher longevity of this lichen species. In experiments where it was compared the levels of PCDD/Fs in younger and older parts of thalli of *X. parietina* it was found that older parts presented higher levels of OCDD (data not shown).

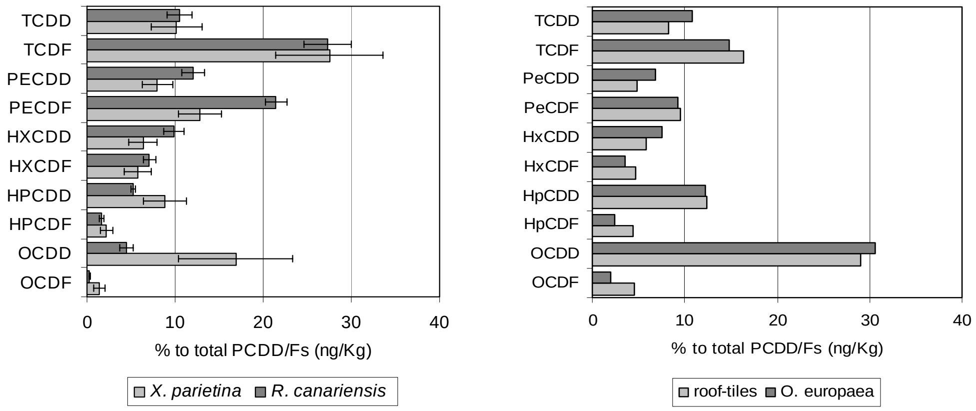

Figure 1. a) PCDD/Fs homologue profile in the lichens *Ramalina canariensis* and *Xanthoria parietina*. Percentage contribution of each homologue to the total PCDD/Fs. N=8. Error bars represent standard deviations. b) PCDD/Fs homologue profile in the lichen *Xanthoria parietina* collected from house roof-tiles and from *Olea europaea* in the same sampling site. Percentage contribution of each homologue to the total PCDD/Fs.

Table 3. Correlations between metal content and percentage contribution of each homologue to the total PCDD/Fs in lichens of the species *X. parietina* and *R. canariensis*. N=8. Marked correlations are significant at $p < .05000$.

		OCDF	OCDD	HPCDF	HPCDD	HXCDF	HXCDD	PECDF	PECDD	TCDF	TCDD
X. parietina	**Zn**	***0.75***	0.46	***0.82***	0.43	***0.79***	-0.11	-0.22	-0.36	-0.46	***-0.73***
	Fe	0.69	0.60	***0.81***	0.53	***0.83***	0.04	-0.51	-0.51	-0.54	***-0.72***
	Mg	-0.11	-0.27	-0.16	-0.25	-0.42	-0.17	0.64	0.21	0.25	0.00
	Mn	0.51	***0.79***	***0.73***	***0.73***	0.69	0.14	-0.67	-0.64	-0.67	***-0.76***
	Ca	0.25	0.40	0.38	0.48	0.41	-0.18	0.08	-0.32	-0.41	-0.58
	K	-0.25	-0.01	-0.08	0.04	0.40	-0.43	0.03	-0.30	0.14	-0.06
	Cu	-0.04	0.00	0.15	0.27	0.33	-0.47	-0.07	0.58	-0.20	-0.04
	Pb	0.59	0.15	0.59	0.31	0.68	-0.33	0.02	0.28	-0.39	-0.40
	Co	0.62	***0.86***	***0.84***	***0.87***	***0.83***	0.11	-0.67	-0.57	***-0.82***	***-0.86***
	Cr	0.29	0.61	0.57	0.66	***0.94***	-0.22	-0.67	-0.50	-0.52	-0.55
R. canariensis	**Zn**	***0.89***	***0.82***	0.28	0.70	***-0.74***	-0.70	***-0.80***	***-0.73***	0.61	0.13
	Fe	-0.19	-0.04	-0.33	-0.10	-0.03	-0.20	-0.10	-0.10	0.31	0.13
	Mg	-0.03	0.01	0.02	-0.14	0.05	-0.08	-0.37	-0.31	0.38	0.41
	Mn	-0.06	-0.15	-0.08	-0.10	0.03	0.25	0.03	0.39	-0.22	-0.10
	Ca	-0.09	-0.11	0.24	-0.24	0.18	0.03	-0.06	-0.23	0.33	-0.14
	K	0.43	0.46	0.11	0.32	-0.30	-0.49	-0.66	-0.35	0.27	0.70
	Cu	0.41	0.37	0.13	0.28	-0.34	-0.42	-0.36	-0.10	0.14	0.19
	Pb	-0.35	-0.37	0.02	-0.44	0.25	0.15	0.42	0.12	0.09	-0.41
	Co	-0.31	-0.02	***-0.89***	-0.06	-0.23	-0.40	-0.07	-0.05	0.27	0.67
	Cr	-0.36	-0.22	-0.37	-0.19	0.15	0.08	0.08	0.15	0.08	-0.02

Bi-plots for the total concentration of PCDD, PCDF and PCDD/Fs are displayed in Figure 2. Even though both species have different types of accumulation of PCDD/Fs, there is a significant correlation between the total concentrations of PCDD, PCDF and PCDD/Fs in the two lichens.

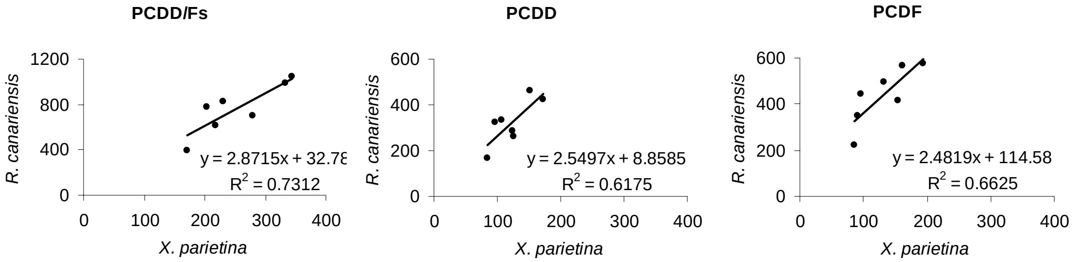

Figure 2. Bi-plots for PCDD/Fs, PCDD and PCDF in the lichens *X. parietina* and *R. canariensis*. N=7 (one outlier excluded).

Homologue profiles between *X. parietina* and *R. canariensis* showed substantial differences. *Xanthoria parietina* showed to be more efficiently interceptor of the most chlorinated PCDD/Fs, which we suggested to be related to a higher interception of particles, whereas *R. canariensis* mainly reflects the less chlorinated PCDD/Fs. Nevertheless we were able to significantly calibrate both species for the PCDD, PCDF and PCDD/F.

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References

1. Martin MH, Coughtrey PJ. *Biological monitoring of heavy metal pollution*. London. Applied Science Publishers 1982:475.
2. Puckett KJ. *Bibliotheca Lichenologica* 1988;30:231-267.
3. Garty J. In: Markert B, editors. *Plants as biomonitors: indicators for heavy metals in the terrestrial environment*. New York: VCH; 1993:193–257.
4. Branquinho C. In: Prasad MNV, editors. *Metals in the environment: analysis by biodiversity*. New York: Marcel Dekker; 2001:117-58.
5. Augusto S, Branquinho C, Pereira MJ, Soares A, Catarino F In: Klumpp, A., Ansel, W., Klumpp, G. (Eds.), *Urban Air Pollution, Bioindication and Environmental Awarenes*. Cuvillier Verlag, Göttingen 2004:67–79.
6. Augusto S, Pinho P, Branquinho C, Pereira MJ, Soares A, Catarino F. *J Atmos Chem* 2004;49:53–65.
7. Augusto S, Branquinho C, Pereira MJ, Soares A. *Int. J. Hyg. Environ.-Health* 2007;210:433–438
8. Augusto S, Catarino F, Branquinho C. *Science of the Total Environment* 2007;377:114–123
9. Garty J. *Crit. Rev. Plant Sci*. 2001;20(4):309–371.
10. Domingo JL, Granero S, Schuhmacher M. *Chemosphere* 2001;43:517–524.
11. Domingo JL, Schuhmacher M, Granero S, Ham DK. *Environ Monit Assess* 2001;69:175–93.