

# ROSEMARY PLANT AS BIO-ACCUMULATOR FOR MONITORING OF PCB CONTAMINATION

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## Introduction

Polychlorobiphenyls (PCBs) are a family of persistent organic pollutants (POPs) ubiquitous in the environment. They can cause cancer, endocrine disruption and neurobehavioral changes in animals and men (EC SCF, 2001), even at very low doses (ATSDR, 2000).

PCBs were intentionally produced by industry as technical mixtures, used as dielectric fluids, organic diluents, plasticizers, adhesives and flame retardants. Although PCBs use and production have been banned in USA in 1970's and in Europe in 1980's, they are still widespread pollutants in air, soil, sediments and biota, especially in industrialized regions.

To monitor the environmental level of these contaminants it could help the availability of biological indicators of PCBs contamination in the environment, since the analytical procedures for PCBs quantification in soil, water, vegetables and animal products is complex and time-consuming.

In this study a great number of vegetables and fruit samples, collected from an industrialized area around the town of Mantua, in the north of Italy, where analysed. The purpose was to assess the level of contamination and the different distribution in the different vegetable species to identify a biological indicator of PCB environmental contamination.

## Material and methods

Samples of various vegetables and fruits (No = 111), including 21 rosemary samples (Tab.1) , were collected between July and October 2005, and June and October 2006 from different areas of the Mantua's district.

All samples, supplied from private's kitchen gardens, were stored at -20 °C until analysis. In accordance with US EPA Method 1613 (1994), the measurement of PCBs was based on the use of  $^{13}\text{C}_{12}$  labelled internal standards (IS). Samples of fruits and vegetables, after washing, homogenization, and lyophilization, were therefore spiked with a mixture of IS of PCBs (12 DL-PCB congeners  $^{13}\text{C}_{12}$  IUPAC Nos. 77,81,126,169,105,114,118,123,156,157,167,189 and 6 NDL-PCB congeners  $^{13}\text{C}_{12}$  IUPAC Nos. 28,52,101,138,153 and 180).

Each sample was subsequently extracted in a Soxhlet apparatus, with a solution of *n*-hexane: acetone 9:1, for 24 hours. After extraction, samples were evaporated to dryness under vacuum, and 15 ml of sulphuric acid (98%) were added and left reacting for at least two hours. Clean-up was carried out overnight with an Extrelut column. After elution with 120 ml *n*-hexane, the samples were concentrated to small volume, and a second purification step was done with an alumina-column, to separate PCBs from PCDDs and PCDFs.

The eluates were evaporated under a gentle stream of nitrogen, and then dissolved with 50  $\mu\text{l}$  of iso-octane. 28 PCB congeners were measured (IUPAC Nos. 28, 52, 81, 77, 101, 99, 95, 110, 123, 118, 114, 105, 126, 151, 149, 146,153, 138, 167, 156, 157, 169, 187, 183, 177, 180, 170, 189) by high resolution gas chromatography-low resolution mass spectrometry (HRGC-LRMS), with a 6890 gas chromatograph coupled with a 5973 MSD mass spectrometer (Agilent, Palo Alto, CA, USA) provided with an auto-sampler 7683 (Agilent, Palo Alto, CA, USA), in the negative chemical ionization (NCI) mode. Methane was used as buffer gas. The carrier gas was helium, with a constant flow of 1 ml/min. An HT-8 (60 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ) capillary column (SGE, Analytical Science, Melbourne, Australia) was used with the following temperature programme: 185 °C for 2 min, 7.5 °C/min increase until 190 °C, 2 °C/min increase until 280 °C, maintained for 10 minutes. The GC-MS was used in the selected ion monitoring (SIM) mode and the monitored ions were  $\text{M}^-$  and  $(\text{M}+2)^-$  for tetra- and penta-CBs and  $(\text{M}+2)^-$  and  $(\text{M}+4)^-$  for hexa- and hepta-CBs. For the limit of detection (LOD) a signal-to-noise ratio of 3:1 was chosen. Concentrations were calculated on whole weight, and the toxic equivalent (TEQ) calculation was based on the 2005 WHO re-evaluation of the toxic equivalent factors (TEFs) (Van den Berg et al., 2006).

## Results and discussion

Table 2 shows TEQ values for dioxin-like PCBs ( $\text{TEQ}_{\text{DL-PCB}}$ ), and non dioxin-like PCB (the so-called "6 indicators",  $\Sigma_{6\text{NDL-PCB}}$ ) and total PCB ( $\Sigma_{28\text{PCB}}$ ) concentrations in all rosemary samples analyzed.

By comparing results in rosemary samples to those in other vegetables analyzed in the present study, a substantial difference in PCB concentrations has been noticed:  $\text{TEQ}_{\text{DL-PCB}}$  values (mean value 0.61 pg/g ww) are ten to hundred times greater in rosemary than in other commodities (mean value 0.01 pg/g ww), and concentrations of  $\Sigma_{6\text{NDL-PCB}}$  (mean value 2.08 ng/g ww) and  $\Sigma_{28\text{PCB}}$  (mean value 4.97 ng/g ww) are ten times greater than in other samples (mean 0.18 and 0.41 ng/g ww, respectively) (Figure 1 and 2).

Comparison of our results with data from the literature shows greater TEQ<sub>DL-PCB</sub> levels in rosemary from this study (0.61 pg/g ww) than in vegetables from some European Countries (0.03-0.12 pg/g whole food) (European Commission, 2001) and from Greece (0.01 pg/g whole weight) (Papadopoulos et al., 2004).

Total PCB levels in rosemary in this study (4.97 ng/g ww) are also higher than in cabbage samples from various European countries, where total PCB concentrations ranging from 0.22 to 0.41 ng/g ww were found (Zuccato et al., 2008). On the contrary, similar PCB concentrations (4.3 ng/g) were found in 1997 by Lovett et al. (1997) in courgettes from Wales and England.

The high PCB levels found in rosemary plant might be ascribable to various factors related to leaf's characteristics. Rosemary leaves are in fact particularly rich in vegetable waxes (Nakamura et al., 1994) that, like previously described in conifers, have the potential to accumulate lipophilic compounds (Reischl et al., 1989).

Furthermore, rosemary is an evergreen plant, with a life span of several years and this might increase its potential to accumulate persistent organic pollutants for prolonged periods of time in comparison to seasonal vegetables.

Another possibility to account for the high PCB levels in rosemary is the short distance between leaves and the ground, where the contaminating PCBs might come from. For instance, also for courgettes, which grow very close to the ground has been hypothesized a direct PCBs adsorption from soil, while other crops, such as leafy vegetables, seem to absorb PCBs mainly by atmospheric deposition (AA Lovett et al., 1997).

Finally, another way of accumulation of PCBs in rosemary could be the re-suspension in air of contaminated soil's particles, which could be then deposited on the leaf's surface.

In a previous study, Di Guardo et al., (2003) hypothesized that conifer needles could be used as passive biomonitors of semi-volatile organic compounds. Similarly, in this study we suggest the use of rosemary as an indicator of PCBs environmental contamination.

The great difference found in PCB levels between rosemary and all other vegetables analysed supports the hypothesis that a continuous POP's accumulation takes place in this kind of plants, both in low contaminated and in high contaminated areas. Levels in rosemary might therefore grossly indicate the extent of the global contamination by PCBs of a given area.

As previously described by Di Guardo et al., (2003) for conifer needles, rosemary and other similar kind of plants might be therefore used as "sentinel" of the environmental contamination by PCBs and other POPs.

## References

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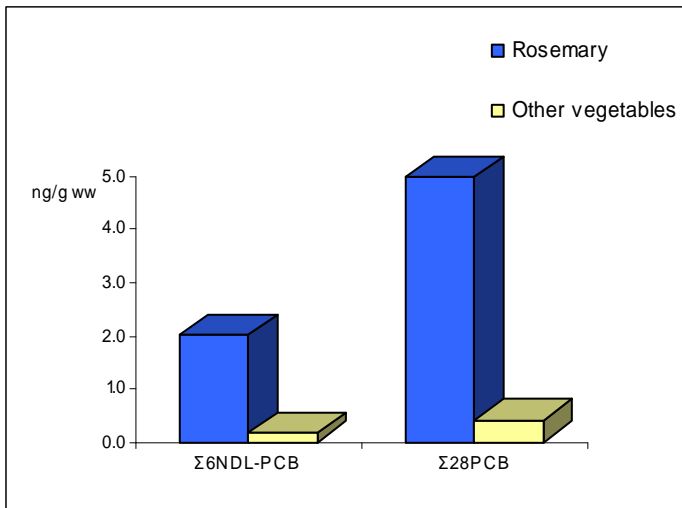


Fig.1: Mean values of  $\Sigma_{6\text{NDL-PCB}}$  and  $\Sigma_{28\text{PCB}}$  in rosemary and other vegetables (ng/g ww)

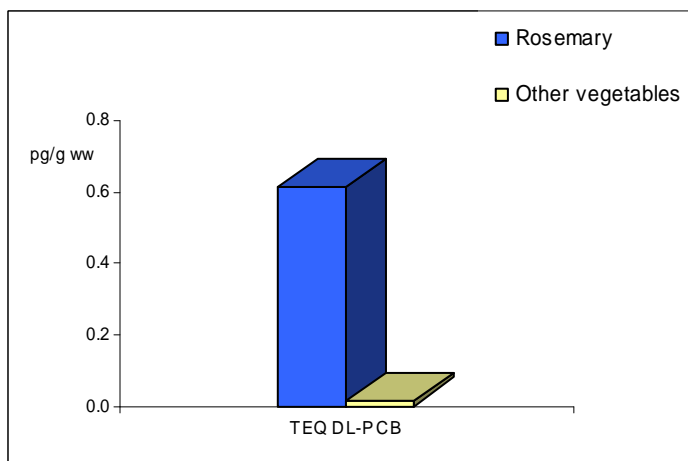


Fig.2: Mean TEQ<sub>DL-PCB</sub> values in rosemary and other vegetables (pg/g ww)

Tab.1: Type and number of vegetables analyzed during this study.

Commodity	Number of samples
rosemary	21
celery, fennel, parsley, lettuce, chicory, radish, savoy, cauliflower, cabbage, broccoli, courgette, cucumber, sage, laurel, aubergine, paprika, tomato, date plum, grape, pomegranate, pear, peach, plum, kiwi	90
Total	111

Tab. 2: TEQ<sub>DL-PCB</sub> values, non dioxin-like PCBs and total-PCB concentrations in rosemary samples analyzed, with mean  $\pm$  SD.

Sample	TEQ <sub>DL-PCB</sub> (pg/g ww)	$\Sigma_6$ NDL-PCB (ng/g ww)	$\Sigma_{28}$ PCB (ng/g ww)
34/05	0.27	1.31	2.95
44/05	0.79	3.01	7.43
59/05	0.28	1.44	3.38
60/05	0.27	1.40	3.16
69/05	1.18	4.50	10.13
74/05	0.95	3.07	7.58
75/05	0.91	3.32	7.81
76/05	0.18	1.03	2.30
84/05	0.23	0.90	2.07
92/05	0.77	2.85	6.96
101/05	1.40	3.96	8.94
7/06	0.44	1.54	4.02
9/06	0.40	1.80	4.70
10/06	0.29	1.20	3.32
16/06	0.26	0.79	2.10
18/06	0.20	0.66	1.82
21/06	0.45	1.12	2.78
37/06	1.31	1.89	4.22
42/06	0.48	2.24	5.08
45/06	0.45	2.02	4.80
47/06	1.34	3.50	8.90
<b>MEAN</b>	<b>0.61</b>	<b>2.08</b>	<b>4.97</b>
SD	0.41	1.13	2.60