Available online at www.sciencedirect.com
ScienceDirect
Environmental Pollution 151 (2008) 341–351
ENVIRONMENTAL POLLUTION
www.elsevier.com/locate/envpol

Correlating element atmospheric deposition and cancer mortality in Portugal: Data handling and preliminary results

S. Sarmento [a,b,*], H.Th. Wolterbeek [a], T.G. Verburg [a], M.C. Freitas [b]

[a] Department of Radiation, Radionuclides & Reactors, Section RIH (Radiation and Isotopes in Health), Faculty of Applied Sciences, Technical University of Delft, Mekelweg 15, 2629 JB Delft, The Netherlands

[b] Instituto Tecnológico e Nuclear, Departamento Reactor, Estrada Nacional 10, 2686-953 Sacavém, Portugal

Received 15 June 2007; accepted 17 June 2007

Relationship between chemical elements and mortality from neoplasms in the Portuguese population and the effect of confounders.

Abstract

This study, framed within geographical epidemiology, presents preliminary findings concerning the association between the concentrations of chemical elements obtained through atmospheric biomonitoring with lichens and cancer mortality in the Portuguese population. Exploratory analyses were performed to identify potential confounders for the relationships between chemical elements and neoplasm mortality and to assess the extent of their interference. The results of this study highlight some methodological and conceptual difficulties inherent to observational and geographical studies, in the specific context of the Portuguese population, and the challenge posed by the large numbers of pollutants considered.

Keywords: Atmospheric biomonitoring; Lichens; Geographical epidemiology; Confounding; Neoplasms

1. Introduction

“What is it in air pollution, and particulate matter (PM) in particular, that might be mediating the observed acute and chronic health effects?” has been a long-standing question in air pollution epidemiology. Among the numerous hypotheses dedicated to particle size and specific PM constituents is one that is concerned with metal content (Maynard, 2003; HEI Perspectives, 2002; Donaldson and MacNee, 2001; NRC, 1998). Numerous toxicological studies have uncovered potential mechanisms for the toxicity of metal-enriched particulates (Ghio et al., 2002; Ghio and Devlin, 2001) and even more numerous occupational studies have established plausible clinical health endpoints that can be attributed to such exposures (reviewed in IARC Monographs and IRIS EPA).

Similar investigations in the context of environmental exposures have been protracted due to the unavailability of systematic and widespread measurements, with the possible exception of Pb (Brunekreef, 1984). Besides the holistic collection of studies performed in the Utah Valley (reviewed in Ghio, 2004) and occasional studies (Dusseldorp et al., 1995; Lipfert et al., 1988; Lipfert, 1980) it was not until recently that researchers began to perform systematic elemental analyses of PM filters in combination with source apportionment techniques (Thurston et al., 2005; Claiborn et al., 2002; Laden et al., 2000) or to resort to historical measurements (Harrison et al., 2004) with the aim of assessing the impact of chemical elements on human health.

Atmospheric biomonitoring uses organisms such as lichens and mosses to measure air quality because these organisms receive their nutrients exclusively from atmospheric deposition (Szczepaniak and Bizuik, 2003; Garty, 2001). Compared to

* Corresponding author. Department of Radiation, Radionuclides & Reactors, Section RIH (Radiation and Isotopes in Health), Faculty of Applied Sciences, Technical University of Delft, Mekelweg 15, 2629 JB Delft, The Netherlands. Tel.: +351 21 9946127; fax: +351 21 9946130.
E-mail address: ssarmento@itn.pt (S. Sarmento).

0269-7491/$ - see front matter
doi:10.1016/j.envpol.2007.06.039

conventional instrumental monitoring methods, biomonitoring offers unique advantages, including the ability to perform high-density sampling at virtually any desired spatial and temporal scales at low cost and allowing the measurement of a wide range of pollutants (Wolterbeek, 2002; Smodis and Parr, 1999; Katsouyanni and Pershagen, 1997). The successful implementation and the usefulness of atmospheric biomonitoring are reflected in the large number of biomonitoring surveys performed throughout the world at international, national and regional levels (Markert et al., 1996), its widespread use in the identification and characterisation of emission sources (Jeran et al., 1996; Kuik et al., 1993), and more recently its application in the realm of human epidemiology (Wolterbeek and Verburg, 2004; Wappelhorst et al., 2000; Cislaghi and Nimis, 1997).

This study presents exploratory analysis framed within a geographical epidemiological study that uses lichen biomonitoring measurements to investigate whether chemical elements in atmospheric deposition might be associated with the incidence of mortality due to malignant neoplasms in the Portuguese population. The results presented here extend previous correlation-based health studies based on biomonitoring data (Wolterbeek and Verburg, 2004; Wappelhorst et al., 2000) by incorporating a preliminary analysis of potential confounding effects. In essence, this study aims to identify elements that might be associated with mortality, identify and characterise potential risk factors for neoplasm mortality in the Portuguese population, identify potential confounders among those risk factors, and provide a preliminary analysis of the latter's impact on the relationship between mortality and the chemical elements.

2. Materials and methods

2.1. Database of chemical elements

The data on elemental concentrations stems from an atmospheric biomonitoring survey performed in 1993 throughout the territory of Continental Portugal. A more detailed account of the sampling and analysis procedure is provided in Reis (2001) and Freitas et al. (2000, 1999, 1997). In summary, the lichen *Parmelia sulcata* was collected in 228 sampling sites and the samples were analysed by k_0-Instrumental Neutron Activation Analysis (k_0-INAA) and Proton-induced X-ray Emission (PIXE).

For the purpose of the present work, the original database was re-evaluated based on state of the art data quality criteria (EPA QA/G-9, 2000), which led to the identification of potential outliers whose samples were reanalysed by INAA (relative method). Furthermore, the revised database differs from the original database in terms of the selection of the analytical technique used for elements measured by both INAA and PIXE (INAA: Al, As, Br, Ce, Co, Cr, Cs, Eu, Fe, Hf, Hg, I, K, La, Lu, Mg, Mn, Rb, Sb, Sc, Se, Sm, Ta, Tb, Th, Ti, U, V and Zn; PIXE: Ca, Cu, Ni, P, Pb, S, Si, Sr and Zr). The final database consisted of a total of 39 elements.

2.2. Study area

Mortality data were available for the third level of NUTS (Eurostat, Ramon), which partitions Continental Portugal into 28 regions.

In order to harmonise the spatial scale of the exposure measurements (points) with that of the mortality data (surfaces), the concentration of each element in each of the 28 regions was calculated as the arithmetic average of the sampling points falling within the boundaries of each NUTS region (Fig. 1). Statistical interpolation techniques were felt to be inadequate due

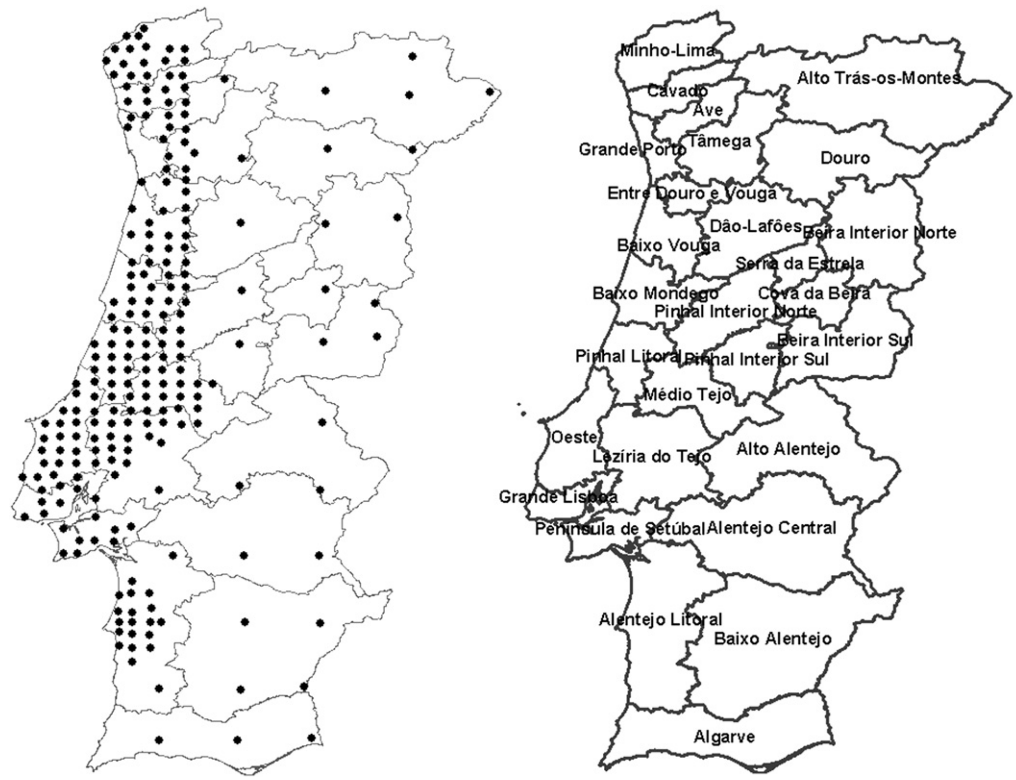

Fig. 1. Maps of the sampling sites (10 × 10 km and 50 × 50 km sampling grid) and NUTS-III regions in Continental Portugal.

to the absence of spatial structure especially in regions covered by the 50×50 km sampling grid (F. Durão, personal communication).

Three NUTS-III regions (Serra da Estrela, Pinhal Interior Sul and Cova da Beira) were excluded from analysis due to the low number of samples within those regions (<2) (Fig. 1). It can be observed from Table 1 that these regions together account for less than 5% of the total area and about 2% of the total population and deaths, and for this reason their exclusion is not believed to constitute a significant source of bias. Table 2 presents, for a selection of elements, the mean regional concentrations and respective 95% confidence intervals.

2.3. Mortality database

The mortality database was provided by the National Statistics Institute (Instituto Nacional de Estatística-Portugal, INE). Gender- and age-adjusted mortality rates due to malignant neoplasms (ICD9: 140–208) (International Classification of Diseases, 1998) were calculated by dividing the number of deaths in each gender and age group by the number of inhabitants in the 1991 Census in the corresponding age and gender groups and expressed as per 1000 inhabitants (INE, 2006).

Since death from cancer is very rare between the ages of 15 and 30 years, the age groups selected for the analyses were restricted to: <15, 30–54 and ≥55 years old.

The place of residence at the time of death was used to ascribe individuals to the different NUTS-III regions and consequently to the different exposure levels.

2.4. Database of ecological risk factors

Information on pertinent ecological risk factors was gathered from the website and publications of the National Statistics Institute (INE, Website; INE, 2006). The variables were selected on the basis of scientific and logical evidence for their potential association with human health and by benchmarking sound health research in the field of air pollution epidemiology. The selection of variables was, in some instances, limited by their unavailability at the required spatial levels and for the required time periods (e.g. income disparity measures) as well as by their unavailability for all regions of interest (e.g. gross domestic product) (Ramos and Rodrigues, 2001).

Preference was given to data referring to a period as close as possible to the year of the biomonitoring survey (1993). Since a census was carried out in 1991, most data refer to this year. However, some important variables were only available for later years (e.g. average expenditure in house buying).

The 18 variables presented here is a shortlist of 26 variables. Preliminary analyses of these variables (results not shown) led to their dismissal for one or more of the following reasons: their ability to reflect the desired constructs was limited (e.g. GDP) (Ramos and Rodrigues, 2001); their correlation with mortality and exposure was not significant (e.g. doctors per 1000 inhabitants, employment in industry); and/or their effect was already captured by other, more reliable variables (e.g. total credit per capita).

The 18 variables selected were grouped into seven general topics: demography, employment, education, economics, fuel sales, land use and nationality (Table 3).

Table 1
Characterisation of the study area: NUTS-III regions and their area, sampling density, resident population and mortality from total malignant neoplasms (ICD9: 140–208)

Regions	Area[b] (km^2)	No. of samples	No. of samples per		No inhabitants[c]	Mortality from malignant neoplasms	
			1000 km^2[b]	100,000 inhabitants[c]		Absolute	Rate per 1000 inhabitants
Minho-Lima	2219	16	7.21	6.4	250,059	6800	27.19
Cávado	1246	8	6.42	2.26	353,267	6471	18.32
Ave	1246	8	6.42	1.72	466,074	8128	17.44
Grande Porto	814	4	4.91	0.34	1,167,800	26,209	22.44
Tâmega	2620	7	2.67	1.37	509,209	8027	15.76
E. Douro e Vouga	862	4	4.64	1.58	252,370	4408	17.47
Douro	4108	2	0.49	0.84	238,695	5368	22.49
Trás-os-Montes	8168	4	0.49	1.7	235,241	5916	25.15
Baixo Vouga	1802	12	6.66	3.42	350,424	7884	22.50
Baixo Mondego	2063	16	7.76	4.87	328,858	7798	23.71
Pinhal Litoral	1742	17	9.76	7.58	224,334	4710	21.00
Pinhal I. Norte	2617	7	2.68	5.02	139,413	4050	29.05
Dão-Lafões	3489	3	0.86	1.06	282,462	6707	23.74
Pinhal I. Sul[a]	1905	1	0.53	1.97	50,801	1401	27.58
Serra da Estrela[a]	868	0	0	0	54,042	1588	29.38
Beira I. Norte	4063	2	0.49	1.69	118,513	3381	28.53
Beira I. Sul	3732	3	0.8	3.7	81,015	2427	29.96
Cova da Beira[a]	1375	1	0.73	1.07	93,097	2402	25.80
Oeste	2220	25	11.26	7.95	314,390	9864	31.38
Grande Lisboa	1376	6	4.36	0.32	1,880,215	50,124	26.66
Setúbal	1525	9	5.9	1.41	640,493	16,218	25.32
Médio Tejo	2306	18	7.81	8.13	221,419	5968	26.95
Lezíria do Tejo	4277	23	5.38	9.87	232,969	6360	27.30
Alentejo Litoral	5255	20	3.81	20.3	98,519	2857	29.00
Alto Alentejo	6230	3	0.48	2.23	134,607	3775	28.04
Alentejo Central	7227	2	0.28	1.15	173,216	4864	28.08
Baixo Alentejo	8504	4	0.47	2.8	143,020	4590	32.09
Algarve	4996	3	0.6	0.88	341,404	10,000	29.29
Total	88,852	228			9,375,926	228,295	
Median			3.25	1.85			26.81

[a] Regions excluded from analyses due to low sampling density.
[b] Source: CAOP 2004, Instituto Geográfico Português and Instituto Nacional de Estatística-Portugal.
[c] Source: Census 1991, Instituto Nacional de Estatística-Portugal.

Table 2
Concentration of seven chemical elements in lichens in 25 NUTS-III regions: mean, 95% confidence intervals and number of samples

Regions	Br			I			Ni		
	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI	*n*
Minho	23.71	18.57–28.86	16	6.90	5.70–8.09	16	3.04	2.67–3.41	16
Cávado	21.59	14.07–29.11	8	4.78	3.56–5.99	8	2.34	1.64–3.04	8
Ave	25.18	17.95–32.42	8	3.61	2.46–4.76	8	4.70	1.93–7.48	8
Porto	33.08	11.94–54.21	4	6.37	0.32–12.42	4	4.18	0.17–8.19	4
Tâmega	21.73	15.43–28.03	7	3.32	2.05–4.60	7	2.80	1.59–4.01	7
E. Douro e Vouga	21.38	16.82–25.93	4	3.52	2.55–4.49	4	2.94	1.26–4.62	4
Douro	9.24	−18.21–36.69	2	2.39	1.69–3.08	2	2.05	−2.77–6.88	2
Trás-os-Montes	10.60	8.12–13.09	4	3.60	2.91–4.28	4	6.45	1.93–10.97	4
Baixo Vouga	25.44	21.09–29.80	12	5.00	3.72–6.27	12	2.28	1.75–2.80	12
Baixo Mondego	21.03	18.04–24.02	16	4.44	3.74–5.14	16	1.67	1.28–2.07	16
Pinhal Litoral	17.76	15.03–20.49	17	5.26	4.05–6.47	17	2.00	1.42–2.58	17
Pinhal I. Norte	12.17	10.92–13.41	7	3.95	3.20–4.70	7	1.88	1.40–2.37	7
Dão Lafões	20.60	11.87–29.33	3	4.95	0.41–9.49	3	3.32	1.10–5.55	3
Beira I. Norte	11.25	0.45–22.05	2	3.06	−2.28–8.40	2	3.34	−10.43–17.10	2
Beira I. Sul	9.02	7.02–11.02	3	3.03	2.01–4.06	3	1.68	1.36–2.00	3
Oeste	29.10	24.98–33.22	24	9.74	8.34–11.14	25	4.26	2.80–5.72	25
Lisboa	26.81	18.25–35.36	6	9.21	7.92–10.50	6	5.96	3.67–8.24	6
Setúbal	21.36	15.88–26.83	9	11.60	8.57–14.64	9	11.99	5.36–18.61	9
Médio Tejo	16.73	12.80–20.66	18	6.76	5.63–7.89	18	4.07	2.85–5.28	18
Lezíria do Tejo	24.73	19.56–29.90	23	7.95	6.84–9.06	23	5.67	3.07–8.28	23
Alentejo Litoral	34.38	24.50–44.27	20	10.02	8.97–11.08	20	6.07	4.79–7.36	20
Alto Alentejo	18.23	4.21–32.25	3	8.00	2.94–13.05	3	3.08	2.31–3.85	3
Alentejo Central	12.75	−10.76–36.26	2	6.14	3.64–8.64	2	3.04	2.43–3.65	2
Baixo Alentejo	16.60	6.09–27.11	4	8.52	2.92–14.11	4	5.76	−1.39–12.91	4
Algarve	19.21	1.85–36.57	3	13.88	−9.08–36.83	3	3.04	1.50–4.57	3

Regions	Pb			S			Sb			V		
	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI	*n*
Minho	25.32	17.24–33.40	16	1931	1756–2107	16	0.17	0.14–0.20	16	7.70	6.13–9.27	16
Cávado	37.63	1.33–73.93	8	2460	1954–2966	8	0.36	0.09–0.63	8	7.99	4.82–11.15	8
Ave	23.60	17.31–29.89	8	2593	1905–3281	8	0.49	0.34–0.64	8	18.45	6.89–30.00	8
Porto	26.72	−7.70–61.15	4	2776	1626–3926	4	0.71	0.33–1.09	4	20.45	8.05–32.85	4
Tâmega	24.57	7.63–41.51	7	2094	1841–2348	7	0.45	0.32–0.57	7	13.12	7.57–18.67	7
Entre Douro e Vouga	16.46	11.49–21.42	4	1961	1210–2712	4	0.32	0.10–0.53	4	12.58	11.61–13.54	4
Douro	12.36	4.03–20.68	2	1215	−195–2625	2	0.14	−0.12–0.39	2	9.05	−1.06–19.15	2
Alto Trás-os-Montes	22.20	−3.29–47.69	4	1626	1392–1860	4	0.22	0.06–0.38	3	19.74	15.61–23.87	4
Baixo Vouga	14.30	11.51–17.10	11	2139	1886–2391	12	0.25	0.20–0.30	12	11.51	8.71–14.31	12
Baixo Mondego	11.26	8.89–13.62	16	1870	1628–2113	16	0.19	0.13–0.26	16	7.92	6.11–9.73	16
Pinhal Litoral	13.34	7.92–18.76	17	1845	1650–2039	17	0.15	0.13–0.18	17	7.79	6.34–9.25	17
Pinhal Interior Norte	11.03	8.58–13.47	7	1425	1237–1613	7	0.17	0.12–0.22	7	8.87	7.16–10.58	7
Dão Lafões	21.74	4.85–38.63	3	2318	−39–4676	3	0.29	0.11–0.47	3	12.84	0.27–25.41	3
Beira Interior Norte	15.22	−26.91–57.34	2	2169	−3091–7429	2	0.15	−0.04–0.34	2	10.70	9.43–11.97	2
Beira Interior Sul	11.46	5.22–17.69	3	1522	1227–1817	3	0.17	0.07–0.27	3	13.05	−0.25–26.34	3
Oeste	12.63	10.11–15.16	25	2363	2227–2499	25	0.21	0.18–0.23	25	15.47	9.80–21.15	25
Lisboa	40.84	20.14–61.53	6	2573	2087–3059	6	0.86	−0.05–77	6	23.08	12.03–34.12	6
Setúbal	49.78	27.40–72.16	9	2778	2564–2991	9	1.10	0.58–1.63	8	50.33	21.66–79.01	9
Médio Tejo	14.27	11.74–16.81	18	1949	1751–2147	18	0.35	0.06–0.64	18	13.80	8.93–18.66	18
Lezíria do Tejo	19.68	13.60–25.77	23	2391	2249–2532	23	0.33	0.26–0.40	23	23.79	10.55–37.03	23
Alentejo Litoral	17.73	11.81–23.64	20	2115	1915–2316	20	0.46	0.34–0.59	19	25.38	19.45–31.31	20
Alto Alentejo	11.09	5.37–16.82	3	1818	1513–2122	3	0.39	−0.21–0.99	3	15.51	1.94–29.07	3
Alentejo Central	10.92	0.19–21.66	2	1568	1245–1891	2	0.19	0.00–0.38	2	12.61	−13.90–39.13	2
Baixo Alentejo	20.00	1.93–38.07	4	1792	1378–2206	4	0.49	0.14–0.84	4	16.43	6.72–26.13	4
Algarve	15.01	10.55–19.47	3	1591	786–2396	3	0.18	0.08–0.27	3	12.51	7.09–17.93	3

2.5. Statistical analyses

Simple and multiple linear regressions were performed with MatLab® version 6 and with SPSS® version 13. The simple linear regressions were performed between: (1) elemental concentrations and mortality in the six age–gender groups; (2) risk factors and mortality in the six age–gender groups; and (3) risk factors and elemental concentrations. Multiple linear regression was performed using just two predictor variables: one element and one potential confounder specific to each mortality–element relationship at a time. Statistical significance was conventioned for *p*-values lower than 5%.

Table 3
The 18 ecological risk factors selected

Category	Name and units	Date	Definition
Demography	Demographic pressure (%)	1991 Census	Sum of the resident population <19 years old and ≥65 years old, divided by the population aged 20–24 years, multiplied by 100.
	Population change (%)	1991 and 2001 census	Difference between the resident population in 2001 and 1991.
Employment	Population employed in agriculture (%)	1991 Census	% Of total employed population in CAE-0 (primary sector).
	Population employed in services (%)		% Of total employed population in CAE-0 (tertiary sector).
	Unemployment rate (%)		% Of active population aged ≥12 years old.
Education	Instruction level (weighed) (%)	1991 Census	Calculated as the sum of the 1st, 2nd and 3rd cycle schooling multiplied by 1, 2 and 3, respectively and divided by the resident population. Refers to the last completed education level.
	Analphabets rate (%)		% Of resident population ≥10 years old who cannot read or write.
Economics	Total bank deposits (1000 Euros)	1991 Census	
	Average expenditure in house buying (Euros)	2001 Census	Refers to classical familiar households used as usual residence and occupied by the proprietor.
	Purchasing power per capita (index)	1995	Indexed to Portugal's mean value, which equals 100.
Fuel sales	Fuel oil sales[b] (tonnes per km^2)	Mean 1999–2001	
	Propane and butane gas sales[b] (tonnes per km^2)		
	Gasoline & Diesel sales[b] (tonnes per km^2)		Does not include leaded gasoline.
Land use	Agricultural land use[a] (%)	1996	% Of total land area.
	Forest land use[a] (%)		
	Urban land use[a] (%)		
Nationality	African nationals (%)	Mean 1991 and 2001 census	% Of the mean resident population in 1991 and 2001 with nationality from Angola, Cape Verde and Mozambique.
	Portuguese nationals (%)		% Of the mean resident population in 1991 and 2001 with Portuguese (single) nationality.

[a] CAOP, 1996, IGP.
[b] CAOP, 2004, IGP.

3. Results

3.1. Simple linear regression between elemental concentrations and total malignant neoplasms

Simple linear regression between mortality rates due to malignant neoplasms (ICD9: 140–208) and the chemical concentrations of 39 chemical elements in 25 regions yielded significant associations for seven elements: Br, I, Ni, Pb, S, Sb and V (results shown in italics in Table 6). Other elements (Hg, Cs, Sr and Zn) also presented significant associations with mortality but these were borderline, less consistent across age–gender groups and, since biological and medical evidence for an association between inhalation exposure to these elements and cancer is either negative or inadequate (IARC Monographs; IRIS EPA), these elements were not considered in further analyses. Mortality in the age group < 15 years old was not significantly associated with any of the 39 elements.

3.2. Simple linear regression between risk factors and total malignant neoplasms

Since this is an observational study, the regions being compared most certainly differ in terms of other health determinants besides the chemical composition of atmospheric deposition. Most of the risk factors considered here presented significant crude associations with mortality, especially in the older age category, and the direction of the associations was consistent across age–gender groups (Table 4). In general, the associations were positive with the exception of those involving: demographic pressure, analphabets rate, employment in agriculture, forest land use and Portuguese nationals.

It is interesting to note that widely publicitised area-level health determinants such as unemployment rate, instruction level and urban land use are not significantly associated with mortality in this population (Willis et al., 2003). In a similar line, the risk factor ‘‘analphabets rate’’, which was originally selected as a complement to ‘‘instruction level’’ to characterise the education of the population, reveals a negative correlation with mortality. This is surprising since several studies in other countries have demonstrated that education is protective due to the greater access to medical care as well as knowledge and power to take attitudes towards protecting health and preventing risks (O'Neill et al., 2003). These observations illustrate the fact that many social, economic and other non-biological health determinants, especially when formulated at the area-level, do not have a clear mechanistic action on disease and if they do, their effects may vary depending on the population under study. For this reason, a great deal of effort must be placed into knowing and understanding the meaning and validity of health determinants in the specific context of the population being studied.

Analysis of the association between analphabets rate and other risk factors (results not shown) reveals that rather than

Table 4
Significance of the regression coefficients when mortality from malignant neoplasms in six age–gender groups is regressed on potential ecological risk factors

Potential risk factors	Mortality rate from total malignant neoplasms					
	$M<15$	M 30–54	$M \geq 55$	$F<15$	F 30–54	$F \geq 55$
Demographic pressure	**n.s.**	**n.s.**	**++**	**n.s.**	**++**	**+++**
Population change	n.s.	n.s.	+	n.s.	+	+
Agriculture employment	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**+**
Services employment	n.s.	n.s.	n.s.	n.s.	n.s.	+
Unemployment rate	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Instruction level	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Analphabets rate	**n.s.**	**n.s.**	**+**	**n.s.**	**n.s.**	**+**
Bank deposits	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
House expenditure	n.s.	+	+	n.s.	n.s.	+
Purchasing power	n.s.	n.s.	++	n.s.	+	+++
Fuel oil sales	n.s.	+	+++	n.s.	+	+++
Gas sales	n.s.	+	++	n.s.	n.s.	++
Gasoline sales	n.s.	+	++	n.s.	n.s.	++
Agricultural land use	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Forest land use	**n.s.**	**n.s.**	**++**	**n.s.**	**n.s.**	**++**
Urban land use	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
African nationals	n.s.	n.s.	++	n.s.	+	++
Portuguese nationals	**n.s.**	**+**	**++**	**n.s.**	**++**	**++**

Row shadings indicate the general grouping of individual risk factors into seven categories: demographic, employment, education, economics, fuel sales, land use and nationality. Please refer to Table 3 for a more detailed description of the risk factors. **Bold** denotes negative regression coefficients. Statistical significance is denoted as: +++: $p<0.001$; ++: $0.001 \leq p < 0.01$; +: $0.01 \leq p < 0.05$; n.s.: $p \geq 0.05$.

indicating the education of the population, as first thought, it appears to indicate the presence of less developed, rural and traditional populations and their associated lifestyles, which in some ways can be regarded as protective towards health (e.g. less stress, traditional Mediterranean diet) but at the same time adverse due to the associated poverty, lower education and isolation.

The absence of an association between urban land use and mortality, on the other hand, appears to be due to problems in the way this variable is operationalised (Eurostat, Metadata). Thus careful understanding of the risk factors is necessary to interpret them correctly and to adequately use them in subsequent, more complex statistical analyses.

Similarly to the simple regression between elements and mortality presented in the previous section the associations between risk factors and mortality rates show a gradient of increasing occurrence and strength with increasing age; there being no risk factors (nor elements) significantly associated with mortality rates in the age group < 15 years old. This pattern suggests that mortality at these young ages is too low to be detected by this type of epidemiological study. In fact, mortality rates per 1000 inhabitants range from 0–0.079 in <15 years old to 6–13 in 30–54 years old and up to 45–131 in ≥55 years old.

3.3. Identification of potential confounders

Some of the risk factors significantly associated with mortality, by virtue of their association with the exposure of interest, may cause a distortion of the effect attributed to the latter on mortality, either by overestimating or underestimating it. This phenomenon, called confounding, is often defined as arising when a risk factor, besides the one of interest is: (1) correlated with both the exposure of interest and the health outcome; (2) not in the causal pathway between exposure and outcome; and (3) not caused by the outcome (Greenland and Morgenstern, 2001; Morgenstern, 1998; Greenland and Robins, 1994).

The aforementioned first criteria of confounding were used to select potential confounders for each element–mortality relationship (Table 5). The sign of the associations in the triangle formed by each combination of mortality–element–potential confounder, considered in Table 5, indicates that in all instances the potential confounding effect is such that it overestimates the effect attributed to the elements (i.e. positive confounding) (Mehio-Sibai et al., 2005). Thus, considering the risk factors analysed, the absence of a crude association between well-established carcinogens such as As and Cr(VI) and the arguably protective Se with mortality could not be due to masking by negative confounding.

Confounder identification requires not only statistical evaluations but also logical reasoning. In this context two issues may be raised: (1) the criteria for a confounder state that it must be correlated with both the exposure of interest and health outcome, however, the correlation with the health outcome must be causal; (2) the second criteria for confounder state that it must not be in the causal pathway between the exposure of interest and the outcome.

Concerning the second issue it is arguable whether fuel sales, and in particular fuel oil sales, can be considered as confounders. Although fuel sales is an indicator of exposures to other substances besides chemical elements, they are a major source of most elements found to be crudely associated with mortality in this study. Although fuel sales cannot be said to be in the causal pathway between elements and mortality, certain elements can certainly be regarded as being in the causal pathway between fuel sales and mortality. This appears to be a case of mathematical coupling rather than of confounding (Tu et al., 2004).

Regarding the first issue, much debate has been dedicated to what exactly is causality (Greenland and Morgenstern, 2001; Pearl, 1995). But this issue becomes more difficult when dealing with variables whose mechanism of action is not known and is a far cry from the mechanistic and objective action of viruses or environmental pollutants.

Table 5
Risk factors that qualify as potential confounders for the relationship between each element and mortality in one of the four age–gender groups (the age group <15 was omitted because no potential confounders were found)

Mortality	EL	Potential confounders
$M \geq 55$	Br	**Demographic pressure**; Population change; **Analphabets rate**; House buy; Purchasing power; Gas sales; Gasoline sales
	I	**Demographic pressure**; Purchasing power; **Forest land use**; African nationals; **Portuguese nationals**
	Ni	**Demographic pressure**; Fuel oil sales; African nationals
	Pb	**Analphabets rate**; House buy; Purchasing power; Fuel oil sales; Gas sales; Gasoline sales; **Forest land use**; African nationals; **Portuguese nationals**
	S	**Demographic pressure**; Population change; **Analphabets rate**; House buy; Purchasing power; Fuel oil sales; Gas sales; Gasoline sales; African nationals
	Sb	**Demographic pressure**; **Analphabets rate**; Purchasing power; Fuel oil sales; Gas sales; Gasoline sales; African nationals
	V	**Demographic pressure**; Purchasing power; Fuel oil sales; African nationals
$F \geq 55$	Br	**Demographic pressure**; Population change; **Agriculture employment**; **Analphabets rate**; House buy; Purchasing power; Gas sales; Gasoline sales
	I	**Demographic pressure**; Services employment; Purchasing power; Forest land use; African nationals; **Portuguese nationals**
	Ni	**Demographic pressure**; Fuel oil sales; African nationals
	Pb	**Analphabets rate**; House buy; Purchasing power; Fuel oil sales; Gas sales; Gasoline sales; **Forest land use**; African nationals; **Portuguese nationals**
	S	**Demographic pressure**; Population change; **Agriculture employment**; **Analphabets rate**; House buy; Purchasing power; Fuel oil sales; Gas sales; Gasoline sales; African nationals
	Sb	**Demographic pressure**; **Agriculture employment**; **Analphabets rate**; Purchasing power; Fuel oil sales; Gas sales; Gasoline sales; African nationals
	V	**Demographic pressure**; Purchasing power; Fuel oil sales; African nationals
M30–54	Br	House buy; Gas sales; Gasoline sales
	I	**Portuguese nationals**
	Ni	Fuel oil sales
	Pb	House buy; Fuel oil sales; Gas sales; Gasoline sales; **Portuguese nationals**
	S	House buy; Fuel oil sales; Gas sales; Gasoline sales
	Sb	Fuel oil sales; Gas sales; Gasoline sales
	V	Fuel oil sales
F30–54	Br	**Demographic pressure**; Population change; Purchasing power
	I	**Demographic pressure**; Purchasing power; African nationals; **Portuguese nationals**
	Ni	**Demographic pressure**; Fuel oil sales; African nationals
	Pb	Purchasing power; Fuel oil sales; African nationals; **Portuguese nationals**
	S	**Demographic pressure**; Population change; Purchasing power; Fuel oil sales; African nationals
	Sb	**Demographic pressure**; Purchasing power; Fuel oil sales; African nationals
	V	**Demographic pressure**; Purchasing power; Fuel oil sales; African nationals

Only those risk factors that are significantly ($p < 0.05$) associated with both mortality and element are shown. Please refer to Table 3 for a more detailed description of the risk factors. **Bold** risk factors denote a negative association with mortality (fortuitously the direction of the association between the risk factors and the elements is the same as for mortality).

3.4. Multiple linear regression with one element and one risk factor as predictor variables

Multiple linear regression analyses were performed for all potential confounders for each element–mortality relationship (see Table 5), by taking predictor variables as one element and one potential confounder at a time. This simple exploratory analysis aims at gaining a better understanding of the underlying relationships between elements and confounders essential for assisting in building more sophisticated regression models with more predictor variables and where issues such as multicollinearity, large errors in regression coefficients and the choice of variable selection method will become more problematic.

As stated before, the potential confounders of each element–mortality relationship are all susceptible of causing an overestimation of the effect of the element, thus incorporation of any of these potential confounders in a multiple regression model will lead to a decrease in the crude regression coefficient of the element and its associated statistical significance. The extent of this decrease is displayed in Table 6.

From Table 6, it can be observed that Br, I and S remain significant predictive variables after adjusting for the vast majority of their respective confounders (exceptions are signalled in grey in Table 6 and involve the relationships between S–$F \geq 55$ – demographic pressure, S–$F \geq 55$ – fuel oil sales and S–$M \geq 55$ – fuel oil sales.). For Ni and V, the opposite is true: these elements lose their predictive ability at the 5% level after adjusting for each of their potential confounders. In the case of Pb and Sb, there is a mix of these two situations, depending on which gender is considered, there being stronger confounders for females than for males. In general, the confounders that tend to override the predictive ability of the elements (grey cells in Table 6) invariably are the following: demographic pressure, purchasing power and fuel oil sales. An alternative method of evaluating the extent of confounding is by analysing the change in estimate of the exposure of interest. This is presented in Table 6 as the percentage decreases in the regression coefficients of the elements after adjustment for the confounder compared to the crude regression coefficient (i.e. without adjustment for the confounder).

Since potential confounders have been defined as being correlated with mortality as well as with the exposure of interest, collinearity between confounders and elements is to be

Table 6
Results of multiple regression taking into account one confounder for each mortality–element relationship

EL	Mort.	Potential confounders	b_{EL}	SE_b	p	VIF	% Decrease in b_{EL}
Br							
	$M \geq 55$	–	*1.033*	*0.291*	*0.002*	*1.00*	–
		Gasoline sales	0.739	0.327	0.034	1.38	*28.5*
		Population change	0.878	0.343	0.018	1.38	*14.9*
		Purchasing power	0.756	0.290	0.016	1.19	*26.8*
		House buy	0.897	0.342	0.016	1.36	*13.1*
		Analphabets rate	0.864	0.315	0.012	1.21	*16.4*
	$F \geq 55$	–	*0.529*	*0.143*	*0.001*	*1.00*	–
		Demographic pressure	0.215	0.154	0.176	1.65	*59.4*
		Gas sales	0.376	0.168	0.035	1.47	*29.0*
		Gasoline sales	0.396	0.162	0.023	1.38	*25.2*
		House buy	0.460	0.168	0.012	1.36	*13.1*
		Population change	0.465	0.169	0.012	1.38	*12.2*
		Analphabets rate	0.459	0.156	0.008	1.21	*13.4*
		Agriculture employment	0.465	0.156	0.007	1.20	*12.2*
	*M*30–54	–	*0.105*	*0.029*	*0.002*	*1.00*	–
		Gas sales	0.087	0.036	0.024	1.47	*16.5*
		Gasoline sales	0.087	0.035	0.020	1.38	*17.0*
		House buy	0.092	0.035	0.015	1.36	*11.7*
	*F*30–54	–	*0.070*	*0.020*	*0.002*	*1.00*	–
		Population change	0.056	0.023	0.026	1.38	*20.1*
		Purchasing power	0.060	0.022	0.011	1.19	*13.9*
I							
	$M \geq 55$	–	*2.640*	*0.634*	*0.000*	*1.00*	–
		Portuguese nationals	2.109	0.815	0.017	1.65	*20.1*
		African nationals	2.023	0.764	0.015	1.51	*23.4*
		Forest land use	1.947	0.706	0.011	1.37	*26.3*
		Demographic pressure	1.972	0.704	0.010	1.36	*25.3*
		Purchasing power	1.995	0.681	0.008	1.30	*24.4*
	$F \geq 55$	–	*1.377*	*0.305*	*0.000*	*1.00*	–
		African nationals	1.050	0.364	0.009	1.51	*23.8*
		Demographic pressure	0.839	0.288	0.008	1.36	*39.1*
		Portuguese nationals	1.218	0.398	0.006	1.65	*11.5*
		Forest land use	1.076	0.345	0.005	1.37	*21.9*
		Purchasing power	0.981	0.309	0.004	1.30	*28.8*
		Services employment	1.360	0.399	0.003	1.63	*1.2*
	*F*30–54	–	*0.189*	*0.042*	*0.000*	*1.00*	–
		Portuguese nationals	0.137	0.052	0.016	1.65	*27.6*
		African nationals	0.170	0.052	0.004	1.51	*10.1*
		Demographic pressure	0.159	0.048	0.003	1.36	*16.0*
		Purchasing power	0.176	0.048	0.001	1.30	*7.1*
Ni							
	$M \geq 55$	–	*2.838*	*0.954*	*0.007*	*1.00*	–
		Fuel oil sales	0.533	1.297	0.685	2.23	*81.2*
		Demographic pressure	1.830	0.972	0.073	1.24	*35.5*
	$F \geq 55$	–	*1.481*	*0.467*	*0.004*	*1.00*	–
		Fuel oil sales	0.411	0.644	0.530	2.23	*72.3*
		Demographic pressure	0.757	0.402	0.073	1.24	*48.9*
Pb							
	$M \geq 55$	–	*0.721*	*0.199*	*0.001*	*1.00*	–
		Gas sales	0.489	0.229	0.044	1.46	*32.2*
		Gasoline sales	0.522	0.225	0.030	1.38	*27.7*
		Purchasing power	0.512	0.211	0.024	1.29	*29.1*
		Analphabets rate	0.615	0.238	0.017	1.41	*14.7*
		Forest land use	0.523	0.199	0.015	1.21	*27.6*
		Portuguese nationals	0.552	0.203	0.013	1.19	*23.5*
		House buy	0.620	0.215	0.009	1.19	*14.0*

Table 6 (*continued*)

EL	Mort.	Potential confounders	b_{EL}	SE_b	p	VIF	% Decrease in b_{EL}
	$F \geq 55$	–	*0.275*	*0.111*	*0.020*	*1.00*	–
		Fuel oil sales	−0.069	0.154	0.658	2.54	*125.0*
		African nationals	0.077	0.138	0.580	1.80	*71.9*
		Gas sales	0.129	0.124	0.312	1.46	*53.3*
		Purchasing power	0.119	0.108	0.280	1.29	*56.7*
		Forest land use	0.163	0.110	0.151	1.21	*40.7*
	$M30–54$	–	*0.062*	*0.022*	*0.010*	*1.00*	–
		House buy	0.050	0.024	0.046	1.19	*19.0*
S							
	$M \geq 55$	–	*0.017*	*0.005*	*0.001*	*1.00*	–
		Fuel oil sales	0.009	0.006	0.151	1.79	*49.1*
		Gasoline sales	0.012	0.006	0.049	1.61	*28.7*
		Demographic pressure	0.012	0.005	0.042	1.45	*31.8*
		Purchasing power	0.012	0.005	0.022	1.28	*28.6*
		Analphabets rate	0.015	0.006	0.019	1.53	*12.8*
		Population change	0.015	0.006	0.014	1.35	*14.5*
		House buy	0.015	0.006	0.013	1.36	*12.5*
		African nationals	0.013	0.005	0.012	1.19	*24.6*
	$F \geq 55$	–	*0.009*	*0.002*	*0.001*	*1.00*	–
		Fuel oil sales	0.005	0.003	0.117	1.79	*46.0*
		Demographic pressure	0.004	0.002	0.074	1.45	*51.0*
		Gasoline sales	0.007	0.003	0.033	1.61	*24.6*
		Purchasing power	0.006	0.002	0.020	1.28	*34.3*
		Analphabets rate	0.008	0.003	0.011	1.53	*7.7*
		House buy	0.008	0.003	0.010	1.36	*12.6*
		Population change	0.008	0.003	0.010	1.35	*12.1*
		African nationals	0.007	0.002	0.009	1.19	*25.8*
		Agriculture employment	0.008	0.003	0.009	1.37	*9.2*
Sb							
	$M \geq 55$	–	*29.104*	*8.557*	*0.002*	*1.00*	–
		Analphabets rate	23.986	9.359	0.018	1.23	*17.6*
	$F \geq 55$	–	*13.946*	*4.353*	*0.004*	*1.00*	–
		Fuel oil sales	−1.774	8.518	0.837	4.40	*112.7*
		Demographic pressure	4.570	4.416	0.312	1.55	*67.2*
		Purchasing power	6.412	5.000	0.213	1.61	*54.0*
		Agriculture employment	11.987	5.225	0.032	1.41	*14.0*
		Analphabets rate	11.582	4.791	0.024	1.23	*17.0*
V							
	$M \geq 55$	–	*0.657*	*0.247*	*0.014*	*1.00*	–
		Fuel oil sales	−0.171	0.374	0.653	2.94	*126.0*
		Demographic pressure	0.342	0.269	0.216	1.39	*48.0*
		Purchasing power	0.413	0.243	0.104	1.19	*37.2*
	$F \geq 55$	–	*0.361*	*0.119*	*0.006*	*1.00*	–
		Fuel oil sales	0.012	0.188	0.949	2.94	*96.7*
		Demographic pressure	0.137	0.111	0.231	1.39	*62.0*
		Purchasing power	0.222	0.111	0.058	1.19	*38.6*

Results are presented only for the cases where the regression coefficient of at least one of the predictor variables (i.e. either the element or the confounder) is significant (p-value < 0.05); in cases where the element is not significant the cells are shaded grey. Shown is the regression coefficient of the element (b_{EL}) its respective standard error (SE_b) significance (p) and Variance Inflation Factor (VIF). In addition the % change in the regression coefficient of the element after adjustment for the confounder compared to the model with only the element (in italics).

expected. Table 6 presents the Variance Inflation Factor (VIF) of the elements' regression coefficients, which in most cases is lower than 1.5. Fuel oil sales tend to present the highest VIF values (close to or higher than 2), indicating the presence of collinearity, which in some cases even results in the inversion of the effect of the element (suppression) (e.g. Sb–$F \geq 55$).

4. Discussion

The apparently simple task of estimating the effect of one variable of interest (element) on a response (mortality) is complicated when inference is based on observational data because of the possibility of bias caused by extraneous variables. The difficulties are enhanced in geographical studies where the mechanisms of action of these extraneous variables on disease are not understood precisely and their effects are dependent on the population under study. Far from a conclusive study, the exploratory analyses presented here highlight the difficulties and pitfalls inherent to observational studies, which are particularly acute when the knowledge of the population is deficient and when several pollutants are being considered (Jorgensen et al., 2007; Lipfert and Wyzga, 1995).

This study found crude associations between Br, I, Ni, Pb, S, Sb and V and mortality due to malignant neoplasms in the Portuguese population. This finding is not consistent with previous correlation studies in the context of atmospheric biomonitoring and neoplasm mortality where crude correlations were found for different elements or in a different direction (Wolterbeek and Verburg, 2004; Wappelhorst et al., 2000). In addition, the identity of these elements (with the exception of Ni) is not consistent with current classifications of human carcinogens by inhalation (IARC Monographs; IRIS EPA). On the other hand, one could conjecture that the absence of crude associations with well-established carcinogens such as As and Cr(VI), whose Unit Risk is actually higher than that of Ni (WHO, 2000; IRIS EPA), could range from threshold effects and inappropriate choice of health outcome to exposure misclassification, to mention just a few. Regarding the choice of health outcome, it is well-known that the main target for cancer after inhalation exposure to these elements is the lung (with a few exceptions). The incidence of mortality due to lung cancer (ICD9: 161–162) in the database of total malignant neoplasms is probably too low to obtain a reliable statistical analysis.

More likely the significant crude association of the seven elements with neoplasm mortality is biased due to confounding. The risk factors analysed here are limited by the absence of any data on lifestyle (e.g. smoking behaviour) and at the individual-level (e.g.: individual instruction level or income) (Greenland and Robins, 1994) owing to their unavailability at the adequate regional level (INS-ONSA, 1998/99). With the revivalism of ecological analyses and the renewed interest in global conditionants of health the question has been raised as to whether individual-level risk factors are really as important as previously thought in studies that compare groups (Steenland et al., 2004; Jerrett et al., 2003; Pearce, 2000; Morgenstern, 1998; Lipfert and Wyzga, 1995). For instance, several health reports have highlighted the fact that traditional risk factors such as lifestyle explain less than 50% of the differences in health across the world (Murray and Lopez, 1996). In this context, some authors believe that socio-economic and other characteristics of the areas exert a strong impact on mortality (Hayes, 2003), and this effect might be partially explained by the way the socio-economic environment in which people live influence lifestyle choices (Steenland et al., 2004).

The risk factors analysed in this study that qualified as potential confounders were all found to be susceptible of causing an overestimation of the effect of the elements if they are not accounted for. This fact eliminates the possibility that the absence of a crude association between other likely carcinogens measured in the biomonitoring survey and mortality might be due to negative confounding.

Although generalisations are difficult to make due to the differences between elements and across mortality categories, this study points to three particularly strong and ubiquitous potential confounders: fuel oil sales, demographic pressure and purchasing power. After adjusting for these confounders most elements' predictive ability was lost at the 5% significance level. Most other confounders did not cause a significant drop in the predictive ability of the elements but the decrease in the elements' regression coefficients was high (~20 to 40%).

5. Conclusion

The possibility offered by biomonitoring methods of measurement of a wide range of pollutants offers the opportunity to investigate whether specific pollutants might be responsible for the health effects observed in air pollution studies. However, since several pollutants tend to be correlated with each other due to common sources, as was the case with the seven elements (results not shown) found to be crudely associated with mortality in this study and since certain sources are associated with specific demographic, socio-economic and lifestyle characteristics of the populations (urban/rural/industrial environments) it can become extremely difficult to disentangle the unique effect of all these variables. As Lipfert et al. (1988) pointed out: ''Since concentrations of many air pollutants are often collinear, this may mean that the pollutant with the least error in estimation of exposure may appear most statistically significant, even when the situation is that a mix of pollutants is responsible for the observed effects''.

Acknowledgments

The authors would like to thank the Fundação para a Ciência e Tecnologia (FCT) in Portugal for the PhD grant, the Instituto Nacional de Estatística-Portugal for the data on mortality and risk factors. We would also like to thank Dr. Fernando Durão from the Instituto Superior Técnico, Lisboa for advice on geostatistical analysis.

References

Brunekreef, B., 1984. The relationship between air lead and blood lead in children: a critical review. Science of the Total Environment 38, 79–123.

Cislaghi, C., Nimis, P.L., 1997. Lichens, air pollution and lung cancer. Nature 387, 463–464.

Claiborn, C.S., Larson, T., Sheppard, L., 2002. Testing the metal hypothesis in Spokane, Washington. Environmental Health Perspectives 110 (4), 547–552.

Donaldson, K., MacNee, W., 2001. Potential mechanisms of adverse pulmonary and cardiovascular effects of particulate air pollution (PM10). International Journal of Hygiene and Environmental Health 203, 411–415.

Dusseldorp, A., Kruize, H., Brunekreef, B., Hofschreuder, P., de Meer, G., van Oudvorst, A.B., 1995. Associations of PM10 and airborne iron with respiratory health of adults living near a steel factory. American Journal of Respiratory and Critical Care Medicine 152, 1932–1939.

EPA QA/G-9, QA00Update. Guidance for Data Quality Assessment, Practical Methods for Data Analysis, July 2000. <www.epa.gov>.

Eurostat Metadata on Land Use. Definition Based on UN-ECE Standard Statistical Classification of Land Use. Available from: <http://europa.eu.int/estatref/info/sdds/en/env/env_land_sm.htm>.

Eurostat, Ramon Classification Server. Nomenclature of Territorial Units for Statistics (NUTS). Available from: <http://ec.europa.eu/comm/eurostat/ramon/nuts/home_regions_en.html>.

Freitas, M.C., Reis, M.A., Alves, L.C., Wolterbeek, H.Th., Verburg, T., Gouveia, M.A., 1997. Biomonitoring of trace-element air pollution in Portugal: qualitative survey. Journal of Radioanalytical and Nuclear Chemistry 217 (1), 21–30.

Freitas, M.C., Reis, M.A., Alves, L.C., Wolterbeek, H.Th., 1999. Distribution in Portugal of some pollutants in the lichen *Parmelia sulcata*. Environmental Pollution 106, 229–235.

Freitas, M.C., Reis, M.A., Alves, L.C., Wolterbeek, H.Th., 2000. Nuclear and analytical techniques in atmospheric trace element studies in Portugal. In: Markert, B., Friese, K. (Eds.), Trace Elements – Their Distribution and Effects in the Environment. Elsevier Science.

Garty, J., 2001. Biomonitoring atmospheric heavy metals with lichens: theory and application. Critical Reviews in Plant Sciences 20 (4), 309–371.

Ghio, A.J., 2004. Biological effects of Utah Valley ambient air particles in humans: a review. Journal of Aerosol Medicine 17 (2), 157–164.

Ghio, A.J., Devlin, R.B., 2001. Inflammatory lung injury after bronchial instillation of air pollution particles. American Journal of Respiratory and Critical Care Medicine 164 (4), 704–708.

Ghio, A.J., Silbajoris, R., Carson, J.L., Samet, J.M., 2002. Biological effects of oil fly ash. Environmental Health Perspectives 110 (Suppl. 1), 89–94.

Greenland, S., Morgenstern, H., 2001. Confounding in health research. Annual Review of Public Health 22, 189–212.

Greenland, S., Robins, J., 1994. Invited commentary: ecologic studies – biases, misconceptions and counterexamples. American Journal of Epidemiology 139, 747–760.

Harrison, R.M., Smith, D.J.T., Kibble, A.J., 2004. What is responsible for the carcinogenicity of PM2.5? Occupational and Environmental Medicine 61, 799–805.

Hayes, M.V., 2003. ‘‘Ecological confounders’’ in the context of spatial analysis of the air pollution mortality relationship. Journal of Toxicology and Environmental Health, Part A 66, 1779–1782.

HEI Perspectives, April 2002. Understanding the Health Effects of Components of the PM Mix: Progress and Next Steps. HEI Publications.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. World Health Organisation, International Agency for Research on Cancer. See pertinent volumes and supplements for each element. Available from: <http://monographs.iarc.fr/ENG/Classification/index.php>.

INE. Website – Instituto Nacional de Estatística – Portugal. Available from: <www.ine.pt>.

INE – Instituto Nacional de Estatística-Portugal, 2006. O País em Números. versão 3.0 CD-Rom. Colecção Estatística em CD-Rom. INE.

INS-ONSA, 1998/99. Inquérito Nacional de Saúde Observatório Nacional de Saúde – Instituto Nacional de Saúde Dr. Ricardo Jorge.

International Classification of Diseases 9. European Shortlist. Final List, August, 1998. Ramon Eurostat's Classification Server. <http://ec.europa.eu/comm/eurostat/ramon/nomenclatures/>.

IRIS, EPA. Integrated Risk Information System, US Environmental Protection Agency. See pertinent volumes and supplements for each element. Available from: <www.epa.gov/iris/>.

Jeran, Z., Jacimovic, R., Batic, F., Smodis, B., Wolterbeek, H.Th., 1996. Atmospheric heavy metal pollution in Slovenia derived from results for epiphytic lichens. Analytical and Bioanalytical Chemistry 354 (5–6), 681–687.

Jerrett, M., Burnett, R.T., Willis, A., Krewski, D., Goldberg, M.S., DeLuca, P., Finkelstein, N., 2003. Spatial analysis of the air pollution–mortality relationship in the context of ecologic confounders. Journal of Toxicology and Environmental Health, Part A 66, 1735–1777.

Jorgensen, E.B., Keiding, N., Grandjean, P., Weihe, P., 2007. Confounder selection in environmental epidemiology: assessment of health effects of prenatal mercury exposure. Annals of Epidemiology 17 (1), 27–35.

Katsouyanni, K., Pershagen, G., 1997. Ambient air pollution exposure and cancer. Cancer Causes and Control 8, 248–291.

Kuik, P., Sloof, J.E., Wolterbeek, H.Th., 1993. Application of Monte Carlo-assisted factor analysis to large sets of environmental pollution data. Atmospheric Environment Part A 27 (13), 1975–1983.

Laden, F., Neas, L.M., Dockery, D.W., Schwartz, J., 2000. Association of fine particulate matter from different sources with daily mortality in six US cities. Environmental Health Perspectives 108 (10), 941–947.

Lipfert, F.W., 1980. Statistical studies of mortality and air pollution. Multiple regression analyses by causes of death. Science of the Total Environment 16, 165–183.

Lipfert, F.W., Malone, R.G., Daum, M.L., et al. April 1988. A Statistical Study of the Macroepidemiology of Air Pollution and Total Mortality. Report 52122. Brookhaven National Laboratory, Upton, NY 11973.

Lipfert, F.W., Wyzga, R.E., 1995. Air pollution and mortality: issues and uncertainties. Journal of the Air & Waste Management Association 45 (12), 949–966.

Markert, B., Herpin, U., Siewers, U., Berlekamp, J., Lieth, H., 1996. The German heavy metal survey by means of mosses. Science of the Total Environment 182 (1–3), 159–168.

Maynard, R., 2003. Scientific information needs for regulatory decision making. Journal of Toxicology and Environmental Health, Part A 66, 1499–1501.

Mehio-Sibai, A., Feinleib, M., Sibai, T.A., Armenian, H.K., 2005. A positive or negative confounding variable? A simple teaching aid for clinicians and students. Annals of Epidemiology 15, 421–423.

Morgenstern, H., 1998. Ecologic study. In: Armitage, P., Colton, T. (Eds.), Encyclopaedia of Biostatistics, vol. 2. Wiley and Sons, New York, pp. 1255–1276.

Murray, C.J.L., Lopez, A.D., 1996. The Global Burden of Disease. A Comprehensive Assessment of Mortality and Disability from Diseases, Injuries and Risk Factors in 1990 Projected to 2020. Harvard School of Public Health, Boston, MA.

NRC, National Research Council, Committee on Research Priorities for Airborne Particular Matter, 1998. Research Priorities for Airborne Particulate Matter: I. Immediate Priorities and Long-range Research Portfolio. National Academy Press, Washington, DC.

O'Neill, M.S., Jerrett, M., Kawachi, I., Levy, J.I., Cohen, A.J., et al., 2003. Health, wealth and air pollution: advancing theory and methods. Environmental Health Perspectives 111, 1861–1870.

Pearl, J., 1995. Causal diagrams for empirical research. Biometrika 82, 669–710.

Ramos, P.N., Rodrigues, A., 2001. Porque é diferente o PIB per capita das regiões Portuguesas? INE/DRC. Cadernos Regionais-Região Centro No. 13.

Reis, M.A., 2001. Biomonitoring and Assessment of Atmospheric Trace Elements in Portugal. Methods, Response Modeling and Nuclear Analytical Techniques. Ph.D. Aggregation. Technical University of Delft, The Netherlands.

Smodis, B., Parr, R.M., 1999. Biomonitoring of air pollution as exemplified by recent IAEA programs. Biological Trace Element Research 71, 257–266.

Steenland, K., Henley, J., Calle, E., Thun, M., 2004. Individual- and area-level socioeconomic status variables as predictors of mortality in a cohort of 179,383 persons. American Journal of Epidemiology 159 (11), 1047–1056.

Szczepaniak, K., Bizuik, M., 2003. Aspects of the biomonitoring studies using mosses and lichens as indicators of metal pollution. Environmental Research 93, 221–230.

Thurston, G.D., Ito, K., Mar, T., Christensen, W.F., Eatough, D.J., Henry, R.C., Kim, E., Laden, F., Lall, R., Larson, T.V., Liu, H., Neas, L., Pinto, J., Stolzel, M., Suh, H., Hopke, P.K., 2005. Workgroup report: workshop on source apportionment of particulate matter health effects – intercomparison of results and implications. Environmental Health Perspectives 113 (12), 1768–1774.

Tu, Y.-K., Clerehugh, V., Gilthorpe, M.S., 2004. Collinearity in linear regression is a serious problem in oral health research. European Journal of Oral Sciences 112, 389–397.

Wappelhorst, O., Kuhn, I., Oehlmann, J., Markert, B., 2000. Deposition and disease: a moss monitoring project as an approach to ascertaining potential connections. Science of the Total Environment 249, 243–256.

WHO, 2000. Air Quality Guidelines for Europe, second ed. World Health Organisation European Office, Copenhagen.

Willis, A., Krewski, D., Jerrett, M., Goldberg, M.S., Burnett, R.T., 2003. Selection of ecologic covariates in the American Cancer Society Study. Journal of Toxicology and Environmental Health, Part A 66, 1563–1589.

Wolterbeek, H.Th., 2002. Biomonitoring of trace element air pollution: principles, possibilities and perspectives. Environmental Pollution 120, 11–21.

Wolterbeek, H.Th., Verburg, T.G., 2004. Atmospheric metal deposition in a moss data correlation study with mortality and disease in the Netherlands. Science of the Total Environment 319, 53–64.