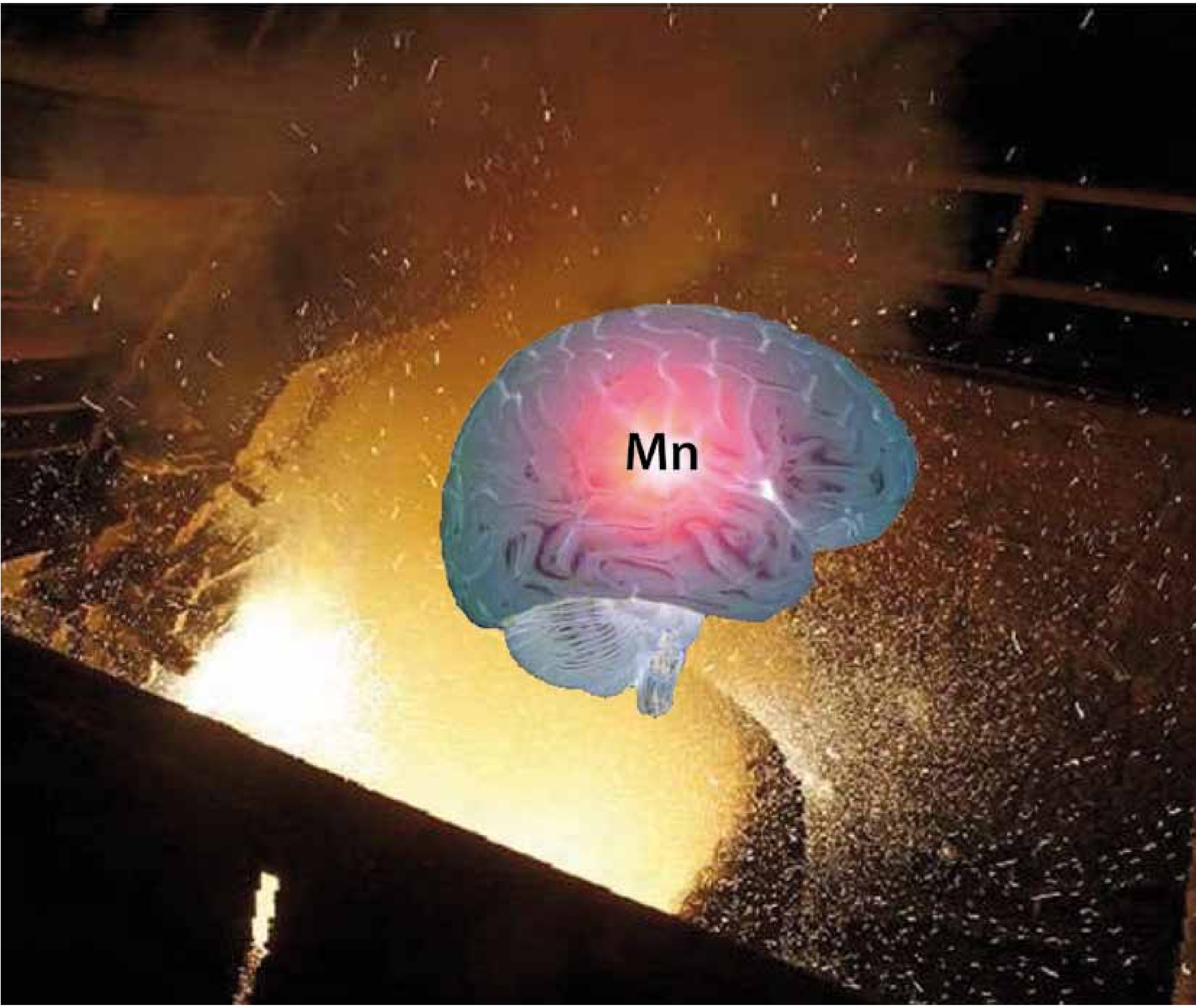
Journal of Environmental Monitoring

Cutting-Edge Research on Environmental Processes & Impacts

www.rsc.org/jem

Volume 11 | Number 9 | September 2009 | Pages 1553–1708



ISSN 1464-0325

RSC Publishing

1464-0325(2009)11:9;1-0

Analysis of settled dust with X-ray Fluorescence for exposure assessment of metals in the province of Brescia, Italy

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Received 1st April 2009, Accepted 1st July 2009
First published as an Advance Article on the web 11th July 2009
DOI: 10.1039/b906430c

Ferroalloy industries have been active for more than a century in the province of Brescia, Northern Italy. Air emission and water discharge have contaminated the environment in the surroundings of four plants with several metals including manganese. The presence of manganese in this region is especially interesting, because of the observed relationship between manganese exposure and Parkinsonism in a previous epidemiological survey. The aim of this study was represented by an initial screening of metal exposure in this area, using a geographic information system. X-ray fluorescence (XRF) was applied to identify heavy metals in deposited dust samples, collected in representative residential households throughout the province. The results were interpreted through a systematic mapping of all municipal districts of the Brescia province. A more frequent distribution of manganese and other metals was observed in the municipalities where the plants were located and differences in the geochemical and anthropogenic origin of metals were discussed, according to the point sources.

Introduction

The ferroalloy industry produces manganese (Mn) based alloys that are used in the steel industry to confer special properties to steel. Minerals containing metals are crushed and melted in an electric furnace in different proportions to obtain the specific alloys. Two fundamental types are used: ferro-Mn and silico-Mn alloys. Emission from this industry causes contamination of air, water and soil with several metals primarily consisting of Mn and iron (Fe), and with the possibility of smaller amounts of lead (Pb) and chromium (Cr).

Occupational and environmental exposure to metals can result in systematic absorption through inhalation and the olfactory pathway and increase the dietary absorption through food and water contamination. Although being an essential element, Mn can exceed the homeostatic range and cause adverse effects for the organism and especially for the central nervous system. Mn accumulates in the globus pallidus, a small area of the basal ganglia, responsible for movement control and mood regulation. Therefore, Mn toxicity targets specifically the motor functions and the well known clinical manifestation of manganism includes Parkinson-like features and psychiatric disorders with marked aggressivity.[1] Historically, this intoxication was observed among miners, industrial workers, and agricultural workers. More recently, concern has been raised about the possible neurodegenerative effects of Mn.[2] Increased prevalence of parkinsonism has been observed among Mn-exposed welders[3,4] and populations living close to industrial sources of Mn pollution and car exhaust gases produced by Methylcyclopentadienyl Mn tricarbonyl (MMT)-based petrol.[5,6]

The possible mechanisms of toxicity underlying Mn-induced parkinsonism are being actively discussed.[7] A recent work[8] showed that inhaled Mn produce morphological and behavioural alterations similar to those observed in Parkinson's diseases (PD) in mice. Therefore, increasing attention and concern has being devoted to Mn environmental exposure.

Environmental impacts of heavy metals are due to their persistence in different matrices also over hundreds to thousands years: they cannot be degraded by any method and may be transferred to humans *via* ingestion, dermal contact, or breathing. They can bio-accumulate in plants and animals and consequently in humans through the food chain.[9] In this respect, the presence of Mn in environmental deposited dust resulting from industrial emissions can represent a risk for human health. Mossetti *et al.*[10] have reported that in the city of Milan soil contribute 15% of PM10. In addition small size particles can derive from larger sized deposited dust and be inhaled due to re-suspension mechanisms. Furthermore to the higher risk associated to finer soil particles because of the possibility of ingestion or inhalation, many soil pollutants are present in this fraction at a higher concentration than in larger size particles due to the higher surface-to-mass ratio.[11]

Manganese was recently found to be highly enriched in fine particles of less than 1 μm resulting from combustion.[12] When inhaled as airborne fine and ultrafine particulate matter, they can be easily transported throughout the alveoli and absorbed into the blood to produce harmful effects elsewhere in the body. They can also be transported directly to the brain through the olfactory pathway, therefore by-passing the blood–brain barrier.[13]

The aim of the present work was to evaluate the environmental impact of neurotoxic metals, and especially of Mn, in the

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province of Brescia. In a previous study[5] an increased prevalence of parkinsonism was observed in the vicinities of Mn-ferroalloy plants located in Valcamonica, a valley in the pre-Alps in the Northern part of this province. Deposited dust can be a useful indicator of historical air pollution: by collecting dust samples from a surface, it is possible to characterize the different pollutants and identify the different anthropogenic and natural sources.[14] This is why deposited dust measurement is a simple and effective way to detect heavy metal exposure from air pollution that may also be available for re-suspension into the atmosphere. The analysis of metals in settled dust deposited in various locations of the Brescian province was used as a first exposure assessment in relation to the distance from the industrial point sources.

To assess the extent of air pollution deposition, and propose preventive intervention, it was also important to investigate whether the metal concentration in deposited particles were related to the natural soil chemistry. Therefore another objective of this work was to differentiate the natural *vs.* anthropogenic source of environmental pollution. Further measurements in air and soil were then performed based on the findings of this current study and will be published elsewhere.

The results of this work will constitute an important basis for further assessment of health endpoint in this area.

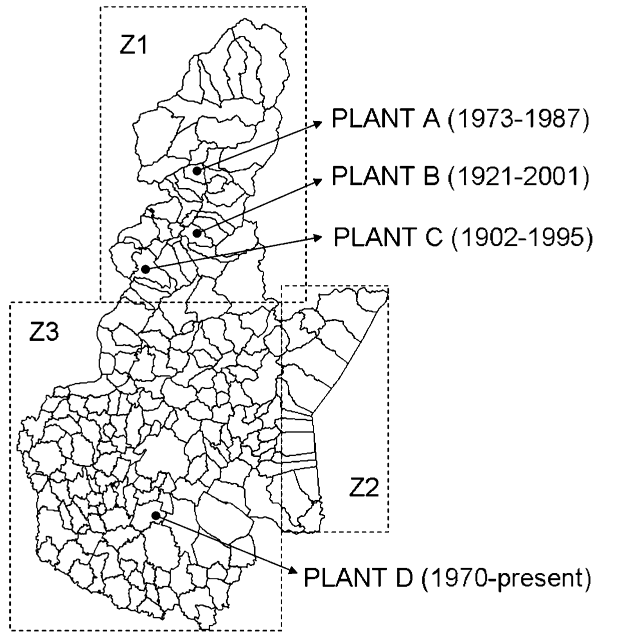

Fig. 1 Map of the Brescia province. All municipal districts are grouped in three areas: Z1 (Valcamonica), Z2 (Garda Lake, as reference zone) and Z3 (the rest of Brescia province). Mn-ferroalloy plants are also reported.

Methods

Area description

The province of Brescia is located within the Lombardia Region in Northern Italy and covers an area of 4,748 km^2, divided into 206 individual municipality districts. According to a 2001 census, the population was 1,088,346 (49% men and 51% women), as shown from the general registry of the Lombardy Region. The municipality of Brescia is the largest in the province and includes a metropolitan area with approximately 200,000 inhabitants. The average number of inhabitants in the other municipalities is 3,610 (range 130–19,979). The province of Brescia is among the most populated of Italy and is well known for its industrial activity which has been highly active since the beginning of the 19th century. Indeed, historical findings have shown evidence of the use of metals dating back to the pre-Roman Iron Age. Three Mn-ferroalloy plants were located in the Northern part of the province, along the pre-Alps Valcamonica valley. This valley runs for about 50 km in the NE-SW direction with an average width of about 3 km and is delimited by mountains of about 3,000 m elevation. Winds blow at the average velocity of 5 km/hr in the direction NE-SW during the day and SW-NE during the night. Therefore, since ferroalloy industries operate 24 hours, atmospheric emissions from the plants have been alternatively transported in both directions NE-SW and SW-NE. The three industries, located in different municipalities about 12 km from each other (Fig. 1), were involved in Mn alloys production from 1973 to 1987 (plant A), from 1921 to 2001 (plant B), and from 1902 to 1995 (plant C) respectively. Plant B was the largest plant, with about 200 workers in its last decades of operation; plant C employed about 100 workers, whereas plant A was the smallest one. In the southern part of the province, there is also a fourth ferroalloy plant, which is still in production and is located in the Padana plains (plant D in Fig. 1). This plant started Mn alloy production in the early 1970s and is still actively employing about 100 workers.

Strategy of exposure assessment

Since ferro-Mn alloy operations ceased completely in 2001 in the pre-Alps study area, deposited dust was identified as an informative matrix of historical exposure for an initial exposure assessment of metals in the area.[14] Coarse road sediments exhibit a relatively low bioavailability of metals but are less than 5% of the total concentration whereas the suspended and subsequently deposited dusts shows increasing potential bio-availability, in the following order Zn > Mn > Cu > Pb > Fe.[15] Bioavailability is due in part to particle size as well as to chemical form (*e.g.* Mn oxides are less soluble and thus less bioavailable than Mn salts).

This is the first work aimed at characterizing the pollution of heavy metals in the province of Brescia, and especially of Mn in the area of Valcamonica. Because of the large size of the entire territory, only one sample was collected for each of the 206 municipal districts, to explore preliminary quantitative data and to discuss their differences. Further measurement of metal concentrations in soil, water and airborne particles will be planned based on the results of this first-step screening, with the intention of further detailing the exposure assessment in the areas that resulted in higher levels in this first step.

Data collection and analysis

Outdoor settled dust was sampled and collected by brushing the marble window sills on the ground floor of houses located in the residential areas of each municipality. The sampling period went from the months of July to December 2004. Samples were collected in vials, previously verified to be metal-free, then sifted with 0.1 mm mesh screens to eliminate the large-sized environmental particles and reduce matrix effect.[16] Each sampling was geo-referenced to allow spatial analysis.

The metal analysis was performed with Energy Dispersion X-ray Fluorescence (EDXRF), using polycarbonate cuvettes and according to the following irradiation parameters: Mo tube - Si(Li) detector - Rh-thin filter - measurements were performed under vacuum - tube tension 20 kV - voltage 80 mA - acquisition time 100 s - calibration with Al–Cu standard.[5]

X-ray fluorescence (XRF) is an interesting and easy to handle technique to evaluate metals present in particle samples. It shows several advantages,[17] mainly because it has element-specific detection capacity, while needing almost no sample pre-treatment. In addition, the multi-element characteristic of this technique allows a fast and useful data analysis. The exposure assessment of heavy metal in this environment was considered as an important prerequisite for planning of further studies on health impacts.

A total number of 15 elements were considered in the ED-XRF analysis, including calcium (Ca), aluminium (Al), silicon (Si), potassium (K), sulfur (S), titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), lead (Pb) and bromine (Br). The percent of metal composition in each dust sample was calculated to obtain rapid and suitable quantitative information, and to compare the exposure levels among the different geographical areas of the territory according to a standardized screening approach.[18] Data were entered into an electronic database and analyzed with descriptive spatial analysis using digital maps of the province (see Fig. 2 for spatial distribution of Al, Ca, Cu, Fe, Mn, Si and Zn).

To further analyse the elements distribution throughout the province, we have subdivided the entire province with its 206 municipalities into three zones: Valcamonica as Zone 1; the Garda Lake, a reference area because of its limited industrial activity, as Zone 2, and the rest of Brescia province at intermediate level of industrial activity as Zone 3 (see Fig. 1). The selection of these different areas stems from a previous finding of a higher prevalence of Parkinsonian disorders in the province of Brescia and, in particular, in Valcamonica, compared to other Italian and European sites.[5]

Results

A total number of 206 samples were collected in the municipalities and analysed by means of ED-XRF.

The cartographical visualisations of the results are reported in Fig. 2, that show the element concentrations, as displayed in colour maps to visually identify their spatial distribution. The first observation from the visual inspection of the maps, is that Mn shows higher levels in some areas, as identified in Fig. 1, that correspond to the ferroalloy plant location and contiguous areas. Moreover, the higher presence of this metal in the north zone (Valcamonica area, identified as Z1), compared with the rest of the province, leaves some open questions about the possible crustal origin of Mn. Concerning other elements, from the visual inspection of their distribution (Fig. 2), we can see that some of them may share the same origin (for example, Al and Si, Fig. 2a and 2f, show similar spatial distributions), but to verify our hypothesis and to identify the actual sources, a comparative data analysis must be performed.

A preliminary data evaluation, considering all samples, points out a different variability in element concentrations. Two metals groups can be identified: a) elements with limited variability (with difference between maximum and minimum percent value less than 7 times): Al, K, Si, Fe, Ti, S and Ca; and b) elements with large variability, reaching values 20 times larger than minimum percent values, including: Cr, V, Cu, Zn, Ni, Pb, Mn and Br.

Fig. 3 shows the percent composition of selected elements, of the first group with a lower range of concentration variability (K, Si, Ti), plotted as a function of Al percentage. Although with some differences, they show a similar trend. The soil in the entire province is generally calcareous with the presence of silicates and clay.[19] The analysis of Si, Ti, and K content as a function of Al show very good correlations, strongly suggesting the same origin of these elements, possibly crustal (*i.e.* from silicates and clays). In addition, the intercept of regression curve of Al and Si is equal to zero, according to the hypothesis of the same source.

A monotone behaviour of Fe content as a function of Al can be found (not shown here), but the correlation is not good, probably because of a double source of this element, crustal and anthropological (metallurgical).

Ca is recognised to be an element strongly related to the crustal nature of the geology of a territory,[20] indeed, considering its concentrations for all samples, it is the most stable element in the settled dust samples. The interesting characteristic of Ca percent is that it does not correlate with other crustal elements. Indeed, in Fig. 2, where the mapping of all elements is related to geographic distributions, the Ca distribution appears to be the opposite of Al. Fig. 4 plots Ca percent *versus* Al, showing a strong inverse correlation. This is in agreement with the nature of the soil in the Brescia province: when settled dust is derived mainly from calcareous soil, Ca contribution increases, compared to Al (and the other related elements). On the contrary, when the silicates and clays contribution increases, the Ca percent decreases.

Concerning S, that is an element with low variability, it does not correlate with the first group of metals, and therefore cannot be associated to the crustal nature of the territory; indeed S is generally related to the atmospheric pollution, produced by several natural (as for example volcanic activity) and anthropogenic activities (as for example combustion processes, metal fusion, *etc.*), often derived from secondary pollution.[20]

Regarding the second group of elements, V and Cr concentrations are highly correlated, suggesting the same (probably metallurgical) origin (Fig. 5), but Cu, Zn, Pb and Ni and Mn data are completely uncorrelated. They cannot be related to the Ca (or Al) percent, excluding a possible crustal origin.

Ni, Cu, Zn and Pb show large variability in all the Brescia province. In particular, in Fig. 2c) and g) we can see a relationship between higher values of Zn and Cu: indeed, the municipal districts with higher concentration of the first metal correspond with those with high values of the second one. This is very likely due to brass metallurgical plants located in these areas.[21] Concerning Ni and Pb, their origin is probably anthropological, but they are in very low quantity (often less than 1%), compared to Mn. In summary these preliminary correlation analyses show that the main source of the elements with low variability Al, K,

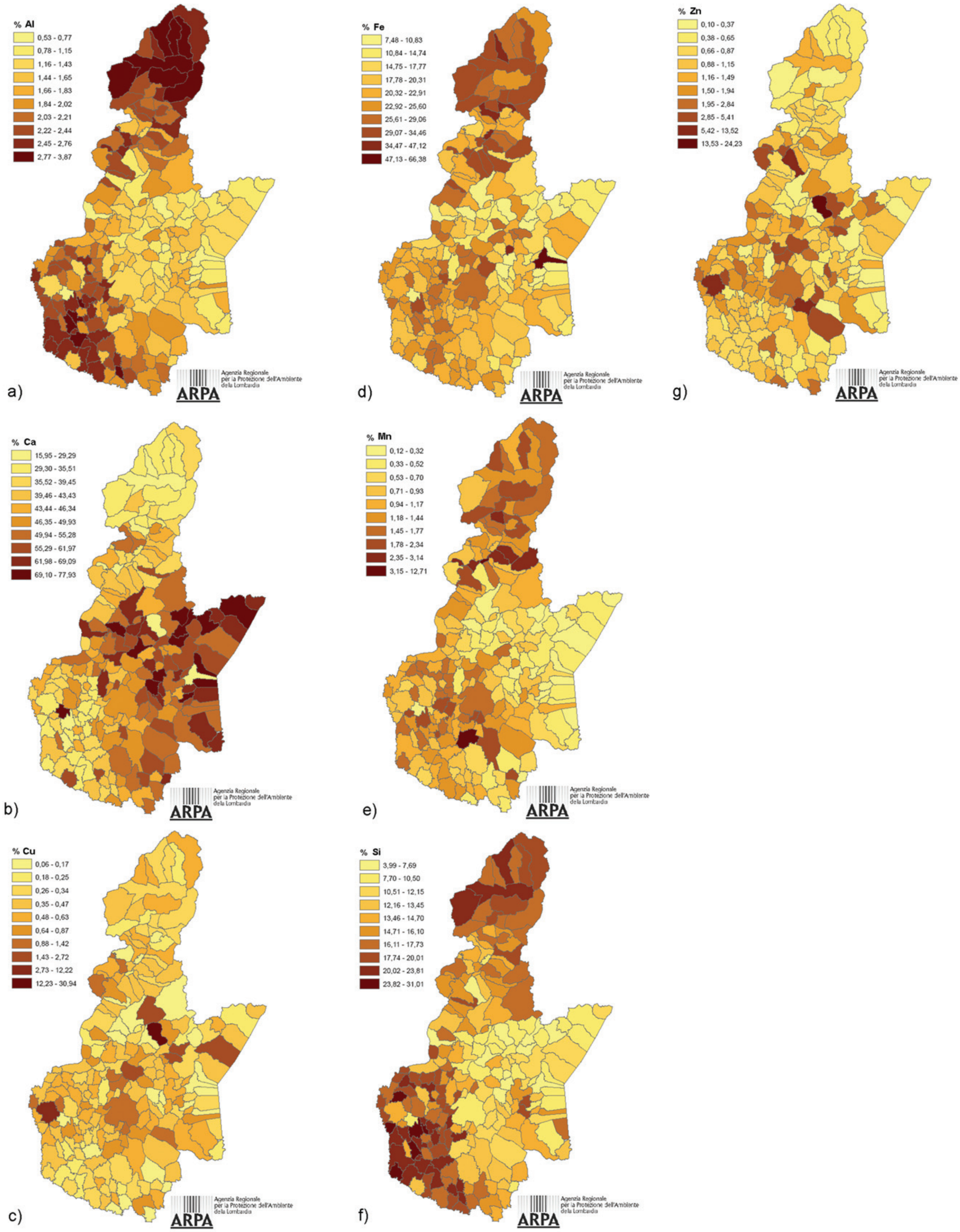

Fig. 2 Cartographical visualisations of selected elements concentrations in settled dust: a) Al, b) Ca, c) Cu, d) Fe, e) Mn, f) Si, g) Zn.

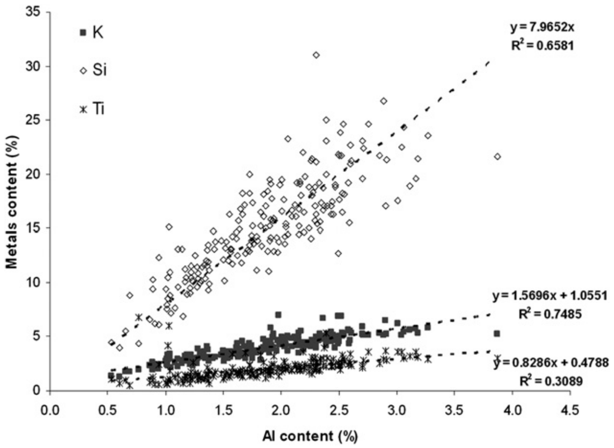

Fig. 3 Correlation between K, Si, Ti and Al concentrations, for settled dust, in all the Brescia province.

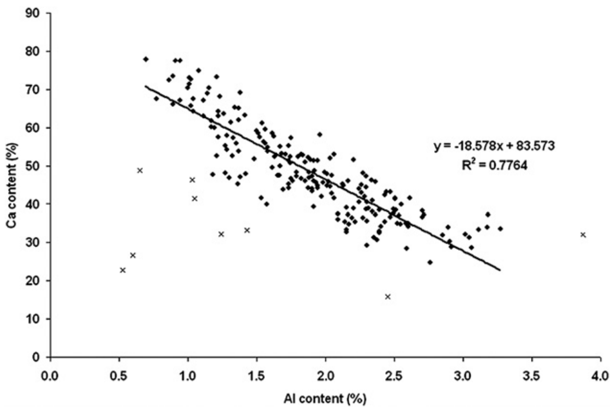

Fig. 4 Correlation between Ca and Al concentrations, for settled dust, in all the Brescia province. X data are not considered in regression analysis.

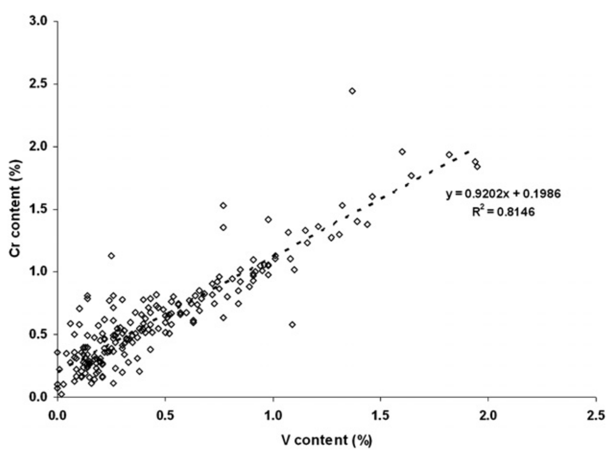

Fig. 5 Correlation between Cr and V concentrations, for settled dust, in all the Brescia province.

Si, Ti, Ca (and Fe) is the soil geochemistry, with the exclusion of S, which is probably related to different natural and anthropological activities also producing secondary air pollution.

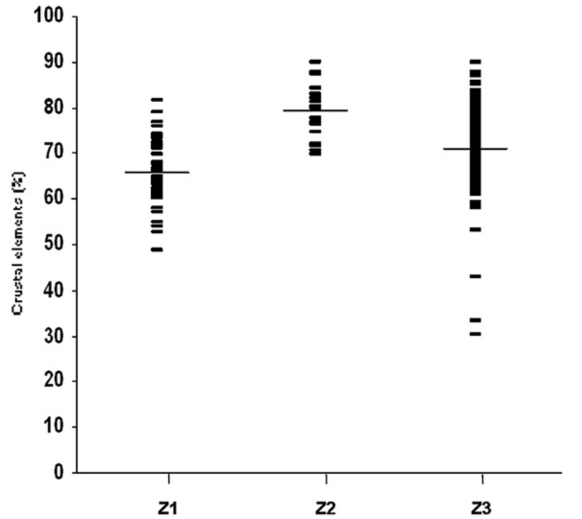

Fig. 6 Sum of crustal elements (Al, Si, K, Ca and Ti contents) for each municipal districts, divided in the three selected areas. The horizontal lines indicate the mean value for each areas.

In addition, as Fig. 6 shows, the composition of the settled dust samples for the areas Z1, Z2 and Z3, considering only crustal elements (Al, Si, K, Ca and Ti), highlights some interesting differences: for Z1, the crustal elements are generally between 49% and 81% of the total with a mean value of 66%; for Z2 they are generally between 70% and 90% with a mean value of 79%, while Z3 shows a settled dust composition of crustal elements between 30% and 90% with a mean value of 71%. These results highlight a high content of other (heavy) metals in settled dust samples for Z1. Zone 2 shows a general lower concentration of heavy metals, when compared to Z1 and Z3 (see Fig. 2): in Z2, where Mn-ferroalloy metallurgical plants were never present, generally the Mn concentration is much lower compared to Z1. In Fig. 7 Al and Mn concentrations are plotted *versus* Fe concentration, considering only the municipal districts of Z2, the control/reference zone. Differently from the other two zones, these metals appear correlated, strongly suggesting the same origin for Fe, Mn and Al (and consequentially Si, Ti and K). This finding suggests that in the Garda area the source of iron and Mn is mainly geochemical, as suggested also by the low variability in the concentrations of these elements.

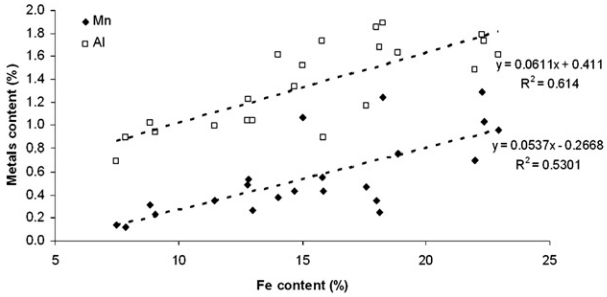

Fig. 7 Correlation between Mn, Al and Fe concentrations, for settled dust, in Z2.

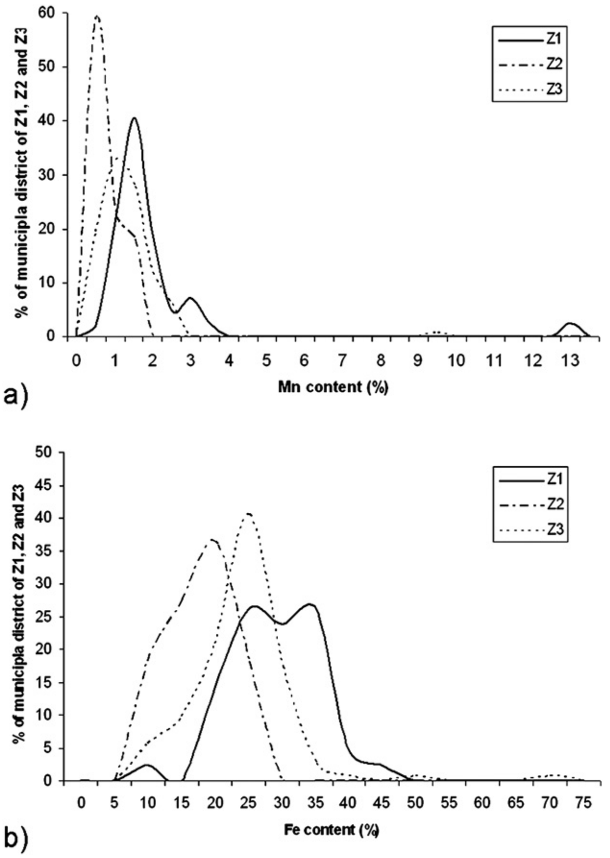

Fig. 8 Distribution of Mn (a) and Fe (b) concentrations in the three selected areas (Z1, Z2 and Z3).

For the entire Brescia province, the metals present in higher quantities (excluding Zn and Cu, which most likely originated from brass industry production and are discussed elsewhere[21]) are Fe and Mn.

Fig. 8 demonstrates the distribution of concentrations of Fe and Mn considering the percent of municipal districts from Z1, Z2 and Z3. As expected, Z1 shows an important amount of municipal districts with high metals concentration. For example, about 10% of the Z1 municipal districts have Mn concentration of about 3% (with one peak at about 13%) and about 25% of municipal districts have Fe concentration of about 35%.

The high levels of these metals are in accordance with the presence of metallurgical Mn- ferroalloy plants (A, B, and C), even if these plants have been closed for several years. This is due to the metals persistence in the environment. Other elements (such as Cr, Ni, Pb, and V) may be related to the anthropological activities, but because of the presence of several industries, with different alloys production, a general correlation between all these elements is not evident; as reported in the first part of the Results section, a clear relationship exists only between Cr and V.

Discussion

These preliminary results about settled dust composition are very important because they have allowed us to obtain the first map of metals concentration distribution in the Brescia province, and also some support for the hypothesis that there are different anthropological and geochemical origins.

From these results it is evident that Z1 contains excessive amounts of heavy metal pollution (in particular Mn) in dust samples. As expected, the Garda Lake area appears to be the less polluted area, with Fe and Mn concentration much lower (about one half) compared to values in Z1. Generally Mn and Fe concentrations are less than 1% and 20% respectively (Fig. 8).

In view of the recently reported results about the bio-availability of metals in suspended dust,[22] in which the Mn is recognized as one of the most dangerous heavy metals, we can conclude that the Z1 population is generally exposed to about twice the Mn concentration in comparison to area Z2. The implications of these findings should be investigated taking into account other parameters of exposure and genetic susceptibility with dietary, air, water and biomarker data. In any case, these preliminary results are quite important, because they support the initial premise about the correlation of Mn with Parkinson's disease (PD) in Valcamonica. Regarding other metals, it may be interesting to investigate possible synergic effects.[23] For example, Fe is an important metal in the regulation of PD: very recently,[24] it was reported that the PD cases had lower total iron binding capacity and transferrin concentrations compared to normal controls, possibly indicating abnormal regulation of iron or iron-related proteins. It was recently suggested[25] that greater lifetime exposure to iron is accompanied by an increased risk of PD. In spite of epidemiological studies investigating associations between occupational or dietary iron exposure and Parkinson's disease that have been inconclusive, exposure to iron was recognized as mostly occurring in conjunction with co-exposures to other metals such as lead, copper, or Mn.[26]

The chemical analysis of a large amount of samples is a very useful method for a rapid exposure assessment of heavy metals and for the identification of critical areas for eventual, further more detailed investigations.

Conclusions

This work, that investigated the heavy metals presence in the largest Italy province, reports and discusses the results of metals concentration in settled dust samples, with particular emphasis on Mn, as a possible cause of PD. The analysis was able to assess the type and extent of metal contamination in different areas: it was possible to determine the most probable metal origins by identifying those with large variations in concentration (Cr, V, Cu, Zn, Ni, Pb, Mn and Br) as anthropogenic in nature and those with low variation in concentration (Al, Si, K, Ca and Ti) as geochemical. In particular, the settled dust in the Valcamonica area showed a higher amount of Mn and Fe concentrations, which can be correlated to the historical presence of ferro-Mn alloy industry activities.

We can also conclude that the increased presence of Mn environmental pollution was demonstrated by this study for a specific area where parkinsonian disease rates have been previously shown to be elevated. From this initial assessment, we can expect that other metals may also represent an environmental problem: the results of this study suggest that the synergistic effects of Mn neurotoxicity in the presence of other metals (for example Fe), should be investigated in more detail.

Environmental exposure assessment should emphasize other factors such as the duration of exposure, diet, lifestyle and societal factors. These factors require additional time and resources for investigation. Based on this initial assessment we have clearly identified the critical zones and at present are working, with the support of the European Union (FP6 project-PHIME) to use a combined approach to quantitative exposure assessment, integrating biological and environmental monitoring. The aim will be to assess a correlation between Mn and neurological effects.

Acknowledgements

This work was partially supported by the EU through its Sixth Framework Programme for RTD (contract no FOOD-CT-2006-016253). It reflects only the author's views. The Community is not liable for any use that may be made of the information contained therein. The authors are grateful to Giovanni Parrinello for useful discussion about data analysis.

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