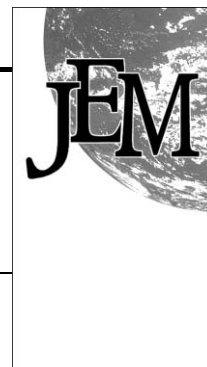


Chemical speciation and sequential extraction of Mn in workroom aerosols: analytical methodology and results from a field study in Mn alloy plants†



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Workers in the Mn alloy producing industry are exposed to aerosols containing a variety of Mn compounds (MnO, MnO₂, Mn₂O₃, Mn₃O₄, FeMn and SiMn). This paper reports a novel four-step chemical speciation/fractionation procedure developed for characterisation of workroom aerosols collected in Mn alloy producing plants. The following components of the aerosol have been quantified: "water soluble" Mn dissolved in 0.01 M ammonium acetate; Mn⁰ and Mn²⁺ dissolved in 25% acetic acid; Mn³⁺ and Mn⁴⁺ dissolved in 0.5% hydroxylamine hydrochloride in 25% acetic acid; and "insoluble" Mn digested in *aqua regia* and hydrofluoric acid. Dissolution of pure Mn compounds with well-defined stoichiometries were essentially complete in the respective leaching steps with detectable amounts of <1% in others. Recoveries of a mixed quality control sample were also acceptable in the range 92–97% for the different oxidation states. The levels measured in the inhalable and respirable fractions in three Mn alloy producing plants were approximately 300 and 35 μg m⁻³ of total Mn, respectively. The most obvious feature of the speciation results is that none of the work areas is characterised by a single Mn contaminant. The predominant oxidation states in the inhalable aerosol fraction are Mn⁰ and Mn²⁺ independent of job functions/departments. The occurrence of insoluble Mn compounds in both the inhalable and respirable aerosol fractions is significantly higher during production of SiMn.

Introduction

Workers in the manganese (Mn) alloy producing industry are exposed to workroom aerosols containing a variety of manganese compounds originating from raw materials, product intermediates and end products. The main manganese ores used as raw materials consist of different manganese oxides (MnO, MnO₂, Mn₂O₃ and Mn₃O₄) where Mn is present in oxidation states II, III and IV. Ferro- and silicomanganese (FeMn, SiMn) with different elemental ratios and carbon contents are the two main products when Mn is present in the metallic form (Mn⁰). In addition, at selected sites the industry produces Mn₂O₃ powder as a side product in the Metal Oxygen Refining process.¹

A host of factors are involved in determining biological responses to occupational exposure of workers.² Important physical and physico-chemical properties and properties of the workroom aerosols containing Mn, which may influence the chemical reactivity at the deposition sites and the fate in the human body, are particle size and shape, solubility and oxidation state. Electron probe microanalysis of individual aerosol particles collected from the workroom air in a Mn alloy plant has shown that none of the particles consists of one single phase or compound, although it was possible to sort the results according to the elemental composition into groups reflecting the main sources and operations in the smelters.³

Owing to the paramagnetic properties of Mn, Boughriet *et al.* characterised particulate forms of Mn compounds in river waters using electron spin resonance spectroscopy.⁴ The speciation of iron and manganese compounds in water particles from a dam was studied by electron spectroscopy for chemical analysis (ESCA).⁵ The chemical nature of Mn-containing

particulates emitted from methylcyclopentadienylmanganese tricarbonyl-added gasoline engines was studied by Mn K-edge X-ray absorption fine structure spectroscopy (XAFS) and X-ray absorption near-edge structure spectroscopy (XANES). Three probable species contained in these particles were identified as Mn₂O₃, MnSO₄ and Mn₅(PO₄)[PO₃(OH)]₂·4H₂O.^{6,7} These individual particle characterisation methodologies have, however, not been applied to workroom air particulates.

Few methodologies have been developed for the bulk speciation of elements present in workroom aerosols. Zatka *et al.*⁸ described a sequential dissolution method for the speciation of nickel in airborne dusts from the nickel-producing industry. This approach was chosen to overcome the intra-particle heterogeneity and encapsulation phenomena common in workroom aerosols and the non-homogeneous distribution of individual species on aerosol filters. No bulk speciation analytical technique capable of identifying and quantifying groups of or individual manganese species present in workroom aerosols has been published. Sequential extraction schemes have been used to assess mobile fractions of a number of elements of ecological importance.⁹ The basic scheme introduced by Tessier *et al.*¹⁰ is commonly applied, and also constitutes the basis for the manganese workroom aerosol schemes presented in this study.

It is currently unclear at which exposure level Mn may cause harm to the workers during production of Mn alloys. As part of an epidemiological study of workers in the Mn alloy industry, a detailed assessment of exposure among the workers was conducted. The novel chemical speciation/fractionation procedure developed in this work for chemical characterisation of work room aerosols was applied (in the measurement of Mn) to a selection of air filters collected during the exposure assessment part of the epidemiological study. In this report the method and selected results from the chemical speciation/fractionation of Mn are presented.

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Experimental

Site description

Ferromanganese and SiMn are the two main products from the Mn alloy industry. In the raw material sites of the smelters the main raw materials, Mn and Fe ores, scrap iron, coke quartz and dolomite, are either stored, mixed or transported. The alloys are produced in electric furnaces, which are fed by a mixture of the raw materials. Finally, the produced alloys are tapped, crushed, screened and packaged for sale. The slag from FeMn furnaces is rich in MnO and is therefore used as raw material in the production of SiMn. A more detailed description of the production steps can be found elsewhere.¹

Air sampling was conducted in all the sectors of three Norwegian Mn alloy producing plants during 1998–1999.

Air sampling

The inhalable aerosol fraction was assessed by employing IOM inhalable personal samplers (SKC Ltd., Blandford Forum, Dorset, UK); each IOM filter cassette was equipped with a 0.8 µm pore-size cellulose-ester membrane filter (25 mm; Millipore, Bedford, MA, USA, AAWP02500). The respirable aerosol fraction was assessed by employing Casella-cyclone samplers (Casella T13026/2, London, England); each cyclone was equipped with a 0.8 µm pore-size cellulose-ester membrane filter (37mm; Millipore, Bedford, MA, USA, AAWP03700). The pumps employed were the in-house, pulsation-free personal units produced by the NIOH (National Institute of Occupational Health, Oslo, Norway). They were operated at a constant flow rate of 2.0 L min⁻¹ (IOM samplers) and 2.2 L min⁻¹ (cyclone samplers), respectively. The flow rate was measured before and after sampling with a calibrated rotameter, type Brooks 2-65 MM (Emerson Electric Co., Hatfield, PA, USA), as a quality control measure.

One hundred volunteer workers from different departments in three different Mn alloy producing plants were recruited; two of the plants produce both FeMn and SiMn and one produces SiMn exclusively. In all, 265 inhalable and 167 respirable full-shift breathing-zone samples were collected among the 100 workers over a three day sampling period.

The proportions (%) of the different components in the respirable and inhalable aerosol fractions were calculated by dividing the actual measured concentration of a given component by the total concentration of Mn. These variables were reasonably normally distributed, and the arithmetic means and corresponding confidence intervals for the proportions were calculated. The skewness of all the distribution curves was below 2.0, as assessed by the Shapiro–Wilk test. Student's *t*-test was applied for calculating statistical differences between groups. The statistical package SPSS[®] version 8.0 was used for the statistical calculations.

Analytical procedures

Background and selection of components. The solubility and oxidation state of Mn are paramount in determining biological activity. In both respiratory tract deposition and clearance of the particles into the gastrointestinal tract, bioavailability of Mn ions is essential. However, little is known of the kinetic differences between soluble Mn (as chloride) and less soluble Mn as oxides. One study comparing MnCl₂ administration and Mn₃O₄ administration indicated that, in general, the concentrations of Mn peaked faster and the tissue peak concentrations were higher for MnCl₂.¹¹ When monitoring workers' exposure to Mn compounds, it may therefore be important to quantify exposure based on the following components.

Component 1: "Water soluble" Mn (ion exchangeable components)–0.01 M ammonium acetate. Solubility in water

influences bioavailability and therefore influences uptake, toxicity and in some cases detoxification.¹ Many reagents have been used to liberate exchangeable metals, ammonium acetate (NH₄Ac) being the most used reagent to liberate Mn in soil and sediment analysis.⁹ In neutral 0.01 M NH₄Ac–aqueous solutions (pH 7.0) it has been shown that this concentration provides a reasonable molar excess of ammonium ions compared with the leachable concentrations of elements from soils.⁹

Component 2: Mn⁰ and Mn²⁺–25% acetic acid. Dilute acetic acid (HAc) has been shown by experiments in our laboratory to be capable of simultaneously dissolving Mn metal, FeMn alloys, MnO and the Mn²⁺ part of the mixed oxide Mn₃O₄. No further attempt was made to try other reagents for further speciation/fractionation of oxidation states 0 and 2+.

Component 3: (Mn³⁺ and Mn⁴⁺)–0.5% hydroxylamine hydrochloride in 25% acetic acid. While the easily-soluble MnO is brought into solution in step 2, the non-dissolved Mn³⁺ part of Mn₃O₄, Mn₂O₃ (Mn³⁺) and MnO₂ (Mn⁴⁺) has been shown to dissolve in a mixture of hydroxylamine hydrochloride and acetic acid.⁹

Component 4: insoluble Mn. Once the first three fractions have been removed, the residual particles should contain silicate-bound Mn or insoluble SiMn alloy. For complete recovery of the insoluble Mn, the residue was dissolved in a mixture of *aqua regia* and hydrofluoric acid under pressure in a Teflon autoclave. This fraction may be considered as non-bioavailable.

Reagents, instrumentation and leaching procedure

The deionised water used throughout was prepared by a Milli Q System (18 MΩ cm). Nitric-, hydrochloric-, hydrofluoric- and acetic acid and hydroxyl ammonium hydrochloride were all of analytical grade quality (Merck, Darmstadt, Germany).

Powdered Mn metal (50 mesh), MnO (60–170 mesh), Mn₂O₃ (325 mesh) and MnO₂ (<10 µm) were all of >>99% purity and obtained from Sigma-Aldrich (Milwaukee, Wisconsin, USA).

Metal Oxygen Refining (MOR) dust (Mn₃O₄) was obtained from the Eramet Mn alloy plant located in Sauda, Norway. This dust contained 96.3% Mn₃O₄ (Lot 203) as reported by the plant laboratory. FeMn and SiMn powders with a Mn content of 80 and 65%, respectively, were obtained from the same plant.

All powders were sieved to a particle size of ≤45 µm (sieve no. 325) before use to ensure that the actual particle size fraction was representative of the inhalable fraction of a realistic workroom aerosol.

A mixture with identical weight ratios (1+1+1+1+1+1) of FeMn, MnO, MnO₂, Mn₂O₃, Mn₃O₄, and SiMn was also prepared for use as a quality control sample.

For the sequential leaching procedure, 50 mL capacity Maxi-Spin polypropylene tubes with 25 mL filter cup inserts equipped with 0.2 µm polyvinylidene fluoride (PVDF) membranes (Part no. 100606, Alltech, Deerfield, IL, USA) were used (Fig. 1). The IOM and cyclone filter samples were placed in the filter cup insert before adding the leaching solution for Component 1. The material collected on the inside surfaces of the IOM aerosol cassette was recovered by extraction using 2 mL of this leaching solution, which were added to the filter cup insert before addition of 8 mL of the rest of the portion for leaching of Component 1.

All centrifugations were made at 2500 rev min⁻¹ with an 8 tube capacity Sigma, Model 4K15 centrifuge (Sigma, Osterode am Harz, Germany). The undissolved residues were dissolved in a mixture of 2 mL of *aqua regia* and 0.5 mL of hydrofluoric acid in Teflon autoclaves with microwave-assisted digestion (MLS 1200, Teflon Container SV140, 10 bar, Milestone,

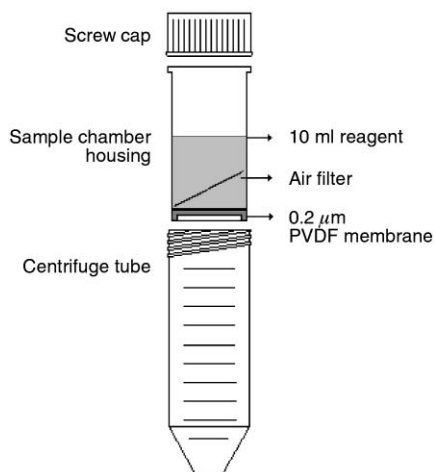


Fig. 1 Polypropylene tubes used in the sequential leaching procedure (see text for details).

Sorisole, Italy) by introducing the whole filter cup insert into the autoclave. Before digestion, 20 µg of beryllium were added to each autoclave as an internal standard. For the measurement of the elemental composition of the leachates, a Perkin–Elmer Optima Model 3000 inductively coupled plasma atomic emission spectrometer was used (Perkin–Elmer, Norwalk, CT, USA).

An in-house, commercially available reference filter material, simulating workroom air concentrations at occupational exposure limit values for individual elements (batches A-2 and B-2) was used to monitor the accuracy and reproducibility of the measurements. For Mn, the measured values were in excellent agreement with the certified value ($< \pm 2\%$) and the day-to-day variation was $\leq 1.5\%$.

Optimisation of leaching conditions

In order to optimise the leaching procedure, 10 mg portions of pure Mn compounds were placed into unused filter cup inserts, which were mounted in the Maxi-Spin tubes, and 10 mL of an appropriate leaching solution were added. The dissolution behaviour throughout the four leaching steps was observed both visually and by measuring Mn in the leachates. The influence of leaching time and temperature on the dissolution was investigated and the optimised conditions are listed in Table 1. For quality control of the leaching procedure, 10 mg

portions of the mixture of pre-sieved pure compounds (particle sizes $< 45 \mu\text{m}$) were analysed routinely.

Results and discussion

The dissolution of the pure stoichiometric Mn compounds was investigated using the optimised leaching conditions (Table 2). Clearly, all compounds, except Mn metal, were quantitatively recovered in the range 94–100% during the expected leaching steps. Dissolution of the Mn compounds was essentially complete in the respective leaching step, with detectable amounts of $< 1\%$ for others. The Mn_3O_4 , which is a mixed $\text{Mn}^{2+}/\text{Mn}^{3+}$ oxide, was also quantitatively recovered as expected in steps 2 and 3. Small amounts of water soluble Mn were detected during fractionation of metallic Mn, which may be due to the smaller particle sizes of the metal powder. It is probable that small amounts of undissolved, nanometer-size particles penetrated through the $0.2 \mu\text{m}$ pore-size membrane filter of the filter insert cup. To prevent oxidation, the Mn metal powder was purchased and stored under an inert atmosphere. It is, however, likely that the particle surfaces may rapidly oxidize when exposed to laboratory air and or during the first water soluble leaching step. This could explain the 5.7% of metallic Mn that was found in the $\text{Mn}^{3+}/\text{Mn}^{4+}$ fraction. The recoveries of the mixed quality control sample were also acceptable, within the range 92–97% for the different oxidation states. The accuracy and reproducibility of all fractionation steps and Mn measurements were assessed continuously by using 10 mg amounts of the in-house prepared mixed quality control sample. During the fractionation and ICP-AES instrumental measurement period (18 days), a total of 18 sub-samples (one each day) were analysed. The recoveries of Mn (percent $\pm s$) for Component 1, Component 2, Component 3 and Component 4 were 107 ± 4 , 92 ± 4 , 107 ± 5 and 94 ± 3 , respectively.

Air measurements

The levels of total Mn measured in the inhalable and respirable fractions in the three Mn alloy plants were approximately 300 and $35 \mu\text{g m}^{-3}$, respectively. The exposure assessment data are available in Norwegian and will be internationally published in detail elsewhere.¹²

The Mn speciation results expressed as a percentage of total Mn in the inhalable and respirable aerosol fractions are summarised in Tables 3 and 4, respectively. The most obvious feature of the speciation results is that none of the work areas during production of either FeMn and or SiMn is characterised

Table 1 Optimised leaching conditions for Mn compounds and alloys

Step	Components	Reagent	Conditions
1	Water soluble Mn	0.01 M ammonium acetate	90 min, 20 °C
2	Mn^0 and Mn^{2+}	25% acetic acid	90 min, 75 °C Microwave-assisted heating
3	Mn^{3+} and Mn^{4+}	0.5% hydroxylamine hydrochloride in 25% acetic acid	90 min, 75 °C Microwave-assisted heating
4	Insoluble Mn	<i>Aqua regia</i> –hydrofluoric acid 4:1	Closed Teflon autoclaves Microwave-assisted heating

Table 2 Leaching recovery in % ($\pm s$) of pure Mn compounds with well-defined stoichiometries, $n=4$

Compound	Component 1 Water soluble	Component 2 Mn^0 , Mn^{2+}	Component 3 Mn^{3+} , Mn^{4+}	Component 4 Insoluble
MnO	< 1	96 ± 1	< 1	< 1
Mn_2O_3	< 1	< 1	94 ± 3	< 1
Mn_3O_4	< 1	100 ± 8 (Mn^{2+})	97 ± 2 (Mn^{3+})	< 1
MnO_2	< 1	< 1	95 ± 1	< 1
Mn	3 ± 0.3	90 ± 3	6 ± 1	< 1
FeMn	< 1	100 ± 1	< 1	< 1
SiMn	< 1	< 1	< 1	98 ± 2
Quality control mixture	< 1	96 ± 6	92 ± 2	97 ± 2

Table 3 Proportions in % of Mn components of total inhalable Mn in the aerosol filters collected during production of FeMn and SiMn

	Mixed FeMn/SiMn plants			SiMn plant			
	<i>N</i> ^a	Mean ^b	95% CI ^c	<i>N</i>	Mean	95% CI	<i>p</i> -values
Components: Water soluble							
Production workers:	91	11	9–13	45	5	3–8	<0.001
Raw material department	27	11	9–14	23	7	2–12	0.09
Smelting department	44	10	7–12	18	4	3–5	0.006
Manufactured product department	11	21	15–26	4	2	0.4–4	0.001
All mechanics/repairmen	40	8	6–9	22	4	3–5	0.004
Components: Mn ⁰ , Mn ²⁺							
Production workers:	91	52	50–55	45	39	33–44	<0.001
Raw material department	27	54	51–58	23	42	34–50	0.003
Smelting operations	44	54	51–57	18	39	29–48	<0.001
Manufactured product department	11	50	46–55	4	23	17–28	<0.001
All mechanics/repairmen	40	53	49–56	22	36	30–42	<0.001
Components: Mn ³⁺ , Mn ⁴⁺							
Production workers:	91	22	18–25	45	9	7–11	<0.001
Raw material department	27	18	12–24	23	9	6–11	0.005
Smelting operations	44	21	18–24	18	11	8–14	<0.001
Manufactured product department	11	12	5–18	4	6	3–8	0.22
All mechanics/repairmen	40	16	13–20	22	9	7–11	0.002
Components: Insoluble							
Production workers:	91	16	13–18	45	47	40–54	<0.001
Raw material department	27	17	12–21	23	43	34–52	<0.001
Smelting operations	44	16	11–21	18	46	33–59	<0.001
Manufactured product department	11	17	11–23	4	70	62–78	<0.001
All mechanics/repairmen	40	24	19–28	22	51	42–60	<0.001

^aNumber of air filters collected. ^bArithmetic mean. ^c95% confidence interval of the mean.

by a single Mn contaminant. In the raw material and manufactured product departments during smelting operations and for all mechanics/repairmen all four Mn components are present. The predominant oxidation states in the inhalable aerosol fraction are Mn⁰ and Mn²⁺ (50–54%) independent of job function/department in the mixed FeMn/SiMn plants, indicating an average 50% occurrence of Mn as FeMn and MnO throughout the production line. It is, however, noticeable that the variation in the occurrence of these two species in the

mixed plants is much reduced when compared with SiMn production only. This is also the case for the respirable aerosol fraction, with a tendency towards higher abundances of Mn⁰ and Mn²⁺ components (Table 4). Soluble Mn components are consistently low in both aerosol fractions (approx. 10–14%); the inhalable fraction in the SiMn plant contains significantly lower amounts of soluble Mn. It is also evident from Tables 3 and 4 that the occurrence of insoluble Mn components in the inhalable and respirable aerosol fractions in all working areas is

Table 4 Proportions of Mn components in % of total respirable Mn in the aerosol filters collected during production of FeMn and SiMn

	Mixed FeMn/SiMn plants			SiMn plant			
	<i>N</i> ^a	Mean ^b	95% CI ^c	<i>N</i>	Mean	95% CI	<i>p</i> -values
Components: Water soluble							
Production workers:	49	11	9–14	48	12	10–14	0.66
Raw material department	15	12	8–15	24	12	9–15	0.95
Smelting operations	24	8	5–12	18	14	8–19	0.07
Manufactured product department	7	21	12–31	6	7	7–8	0.006
All mechanics/repairmen	28	10	8–12	25	10	8–12	0.83
Components: Mn ⁰ , Mn ²⁺							
Production workers:	49	61	56–65	48	47	42–52	<0.001
Raw material department	15	59	54–64	24	51	43–59	0.15
Smelting operations	24	62	53–71	18	47	39–56	0.02
Manufactured product department	7	57	52–62	6	31	26–36	<0.001
All mechanics/repairmen	28	59	55–62	25	47	41–54	0.002
Components: Mn ³⁺ , Mn ⁴⁺							
Production workers:	49	14	10–17	48	10	8–11	0.056
Raw material department	15	17	9–25	24	10	8–13	0.050
Smelting operations	24	12	6–17	18	10	7–12	0.57
Manufactured product department	7	9	0.9–16	6	7	6–8	0.65
All mechanics/repairmen	28	16	13–20	25	9	7–11	0.001
Components: Insoluble							
Production workers:	49	15	9–20	48	31	26–37	<0.001
Raw material department	15	13	6–19	24	27	21–34	0.005
Smelting operations	24	18	7–28	18	29	20–39	0.11
Manufactured product department	7	13	9–17	6	55	48–61	<0.001
All mechanics/repairmen	28	15	11–20	25	34	27–41	<0.001

^aNumber of air filters collected. ^bArithmetic mean. ^c95% confidence interval of the mean.

statistically significantly higher in the SiMn plant. This reflects the general abundance of the biologically very "insoluble" SiMn throughout the production line. There is also a clear tendency for insoluble Mn compounds (SiMn) to be enriched in the inhalable fraction. The particle size can have a significant impact on the solubility, which may explain the significantly reduced difference in exposure levels for water soluble components of the respirable Mn, as presented in Table 4.

Further, it should be emphasized that the leaching scheme, which has been developed for the characterisation of the Mn compounds likely to be present in Mn alloy plants, was developed and validated using pure Mn compounds with well-defined stoichiometries. In an earlier study it was concluded that individual particles collected in the workroom atmosphere of Mn alloy plants consist of a mixture of different phases.² Most of the particles identified contained mainly Fe and/or Mn oxides, oxidised flux components, such as Si, Al, Mg, Ca, Na and K, elemental carbon, or a mixture of all. A recent study of individual particles collected in a nickel smelter showed that the particles were heterogeneous, even on the sub-micrometer scale, with specific areas of various identified amorphous and crystalline phases being of nanometer size.¹³ Whether such complex particles have the same dissolution behaviour as single component particles is unclear and should be studied in further detail.

Concluding remarks

It may be concluded from the present work that it is feasible to fractionate Mn compounds that are known to be present in workroom air of Mn alloy smelters. Because such chemical information is clearly important, speciation analysis of the different health related aerosol fractions is recommended as a routine component of exposure assessments of workers exposed to Mn in this industry.

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References

- 1 D. B. Wellbeloved, P. M. Craven and J. W. Wandby, in *Manganese and Manganese Alloys*, *Ullmann's Encyclopedia of Industrial Chemistry*, ed. B. Elvers, S. Hawkins and G. Schulz, VCH Verlagsgesellschaft, Weinheim, 5th edn., 1990, vol. A16, p. 77.
- 2 E. Nieboer, G. G. Fletcher and Y. Thomassen, *J. Environ. Monit.*, 1999, **1**, 1.
- 3 S. Gunst, S. Weinbruch, M. Wentzel, H. O. Ortner, A. Skogstad, S. Hetland and Y. Thomassen, *J. Environ. Monit.*, 2000, **2**, 65.
- 4 A. Boughriet, B. Ouddane, J. C. Fischer, M. Wartel and G. Leman, *Water Res.*, 1992, **26**(10), 1359.
- 5 M. Zaw and B. Chiswell, *Talanta*, 1995, **2**(1), 27.
- 6 T. Ressler, J. Wong, J. Roos and I. L. Smith, *Environ. Sci. Technol.*, 2000, **34**(6), 950.
- 7 T. Ressler, J. Wong and J. Roos, *J. Synchrotron Radiat.*, 1999, **6**, 656.
- 8 V. J. Zatzka, J. Stuart Warner and D. Maskery, *Environ. Sci. Technol.*, 1991, **26**, 138.
- 9 J. Arunachalam, H. Emons, B. Krasnodebska and C. Mohl, *Sci. Total Environ.*, 1996, **181**, 147.
- 10 A. Tessier, P. G. C. Campbell and B. Bisson, *Anal. Chem.*, 1979, **51**, 844.
- 11 D. M. Drown, S. G. Oberg and R. P. Sharma, *Toxicol. Environ. Health*, 1986, **17**, 201.
- 12 D. Ellingsen, R. Bast-Pettersen, S. Hetland and Y. Thomassen, STAMI-Report No. 6-2000, National Institute of Occupational Health, Oslo, Norway.
- 13 S. Weinbruch, *et al.*, presented at *ENVIROMIN 2001*, Skukuza, Kruger National Park, South Africa, 14–18 July 2001.