

Please cite the Published Version

de Oliveira, Andrea, Polezer, Gabriela, Godoi, Ricardo HM, Vermaak, Sanja Potgieter, de Souza, Rodrigo AF, Andreoli, Rita V and Yamamoto, Carlos I (2020) EXPRESS: Atomic Absorption Spectrometry Methods to Access the Metal Solubility of Aerosols in Artificial Lung Fluid. *Applied Spectroscopy*, 74 (8). pp. 932-939. ISSN 0003-7028

DOI: <https://doi.org/10.1177/0003702820906422>

Publisher: SAGE Publications

Version: Accepted Version

Downloaded from: <https://e-space.mmu.ac.uk/625133/>

Usage rights: © In Copyright

Additional Information: This is an Author Accepted Manuscript of an article published in *Applied Spectroscopy* by SAGE.

Enquiries:

If you have questions about this document, contact openresearch@mmu.ac.uk. Please include the URL of the record in e-space. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from <https://www.mmu.ac.uk/library/using-the-library/policies-and-guidelines>)

Atomic Absorption Spectrometry Methods to Access the Metal Solubility of Aerosols in Artificial Lung Fluid

Gabriela Polezer^a, Ricardo H. M. Godoi^a, Sanja Potgieter-Vermaak^b, Rodrigo A. F. de Souza^c, Rita V. Andreoli^c, Carlos I. Yamamoto^d, Andrea Oliveira^{*,e}

^a Environmental Engineering Department, Federal University of Paraná, Curitiba, PR, Brazil;

^b Ecology and Environment Research Centre, Department of Natural Sciences, Manchester Metropolitan University, Manchester M1 5GD, UK;

^c State University of Amazonas, Meteorology Department, Manaus, Brazil;

^d Chemical Engineering Department, Federal University of Paraná, Curitiba, PR, Brazil;

^e Chemistry Department, Federal University of Paraná, Curitiba, PR, Brazil.

Abstract

Recent studies to quantify the health risks that fine particulate matter with an aerodynamic less than 2.5 micrometers (PM_{2.5}) pose use in vitro approaches. One of these approaches is to incubate PM_{2.5} in artificial lysosomal fluid for a given period at body temperature. These body fluids used have a high ionic strength and as such can be challenging samples to analyze with atomic spectroscopy techniques. As PM_{2.5} is a primary health hazard because it is tiny enough to penetrate deep into the lungs and could, in addition, dissolve in the lung fluid it is important to quantify elements of toxic and/or carcinogenic concerns, reliably and accurately. Sophisticated instrumentation and expensive pre-treatment of challenging samples are not always available, especially in developing countries. To evaluate the applicability of GFAAS without Zeeman correction capability to detect trace quantities of heavy metals leached from PM_{2.5} on to artificial lung fluid, uni- and multivariate approaches have been used for optimization purposes.

The limits of quantification, LOQ, obtained by the optimized method were: 2 µg L⁻¹ (Cu), 3 µg L⁻¹ (Cr), 1 µg L⁻¹ (Mn) and 10 µg L⁻¹ (Pb). The addition/recovery experiments had a mean accuracy of: (Cu) 99 ± 7%; 110 ± 8% (Cr); 95 ± 9% (Mn) and 96 ± 11% (Pb). The average soluble fractions of PM_{2.5} incubated in artificial lysosomal fluid (ALF) for 1 hour were: 1.2 ± 0.01 ng m⁻³ Cu, 0.4 ± 0.01 ng m⁻³ Cr, 0.6 ± 0.01 ng m⁻³ Mn and 4.8 ± 0.03 ng m⁻³ Pb. Using historical elemental

averages of PM_{2.5} in Curitiba (Cu 3.3 ng m⁻³, Cr 2.1 ng m⁻³, Mn 6.1 ng m⁻³, Pb 21 ng m⁻³), the percentage bioaccessibility were determined to be Cu 38%; Cr 20%; Mn 10%; and Pb 23%. The elemental values of the atmospheric soluble fraction of Cu, Cr and, Mn were below the inhalation risk concentrations. However, for Pb, the atmospheric soluble fraction exceeded the inhalation unit risk of 0.012 ng m⁻³.

This robust and straightforward GF AAS method is pivotal for low and middle-income countries where most air pollution adverse effects occur and established **lower-cost** technologies are **likely** unavailable.

Keywords: PM_{2.5}, bioaccessibility, artificial lysosomal fluid, inorganic elements, GFAAS.

*Corresponding author: Andrea Oliveira Tel: +55 (41) 3361-3182

e-mail address: aoliveira@quimica.ufpr.br

Introduction

Aerosol particles comprise organic and inorganic components, some of which could be considered toxic and/or carcinogenic¹⁻⁸ leading to diverse health effects, including cardiovascular, pulmonary diseases and of late, stroke.⁹ These effects are related to particle size and composition of particulate matter,¹⁰ as the inhaled PM_{2.5} can reach the gas exchange region of the alveoli in the lungs upon inhalation exposure. The chemical composition, especially pertaining to the toxic and carcinogenic components, plays a significant part in the risk upon exposure due to its potential mobility in lung fluid (causing a bioaccessible fraction). While the evidence for the differential toxicity of particulate matter is far from conclusive, the nature and action of chemical elements are estimated by their acute inhalation reference concentration (RfC), which is widely published by health and environmental agencies. The inorganic contaminants selected here constitute an important class of pollutants which have received the attention of researchers all over the world, mainly due to their harmful effects on living beings. The RfCs of the elements discussed in this paper are: Cu = 100 µg m⁻³;¹¹ Cr (VI) = 8 ng m⁻³;¹² Mn = 50 ng m⁻³;¹³ and Pb = 0.012 ng m⁻³.¹⁴

Due to solubilized particulate matter's potential adverse health effects, in vitro studies report on the bioaccessibility of PM_{2.5} in artificial lysosomal fluid (ALF).^{15,16} However, as biological fluids produce a complex matrix for analysis, with for example a high salt content¹⁷ it becomes a challenge for the analyst, especially in laboratories where analytical equipment has limitations. In studies involving the bioaccessibility of inorganic elements in ALF, these elements were determined by inductively coupled plasma mass spectrometry and optical emission spectrometry (ICP-MS and ICPOES), during which the ionic strength has to be carefully managed by either severe sample dilution or removal of the ionic species by pre-treatment.¹⁸⁻²⁵ It is also possible to use graphite furnace atomic absorption spectrometry (GFAAS) for the analysis of trace quantities of elements, however, high salt content will also interfere with the analysis, as well as cause high backgrounds. The latter may be addressed with the use of Zeeman Effect background corrector,¹¹

26-27 but remains a problem if the instrument available does not have the Zeeman correction capability.

It is evident that although validated methods for the determination of the metals of interest in this matrix have been published, a robust, precise and accurate method with little or no sample preparation would be beneficial. This paper describes the development, optimization and validation of a method for the quantification of Cu, Cr, Mn, and Pb in a high ionic strength matrix, using GFAAS, without Zeeman correction capabilities.

Materials and Methods

Instrumentation

All quantification measurements of Cu, Cr, Mn and Pb present in the ALF were carried out using a GFAAS (AA 6800, Shimadzu, Japan) equipped with a background correction by deuterium lamp, using pyrolytic graphite tubes longitudinally heated. Argon (99.9% pure) was employed as purge and protective gas. All analyses were performed employing 20 μL of the sample and/or standard and reference solutions and 5 μL of chemical modifier. The wavelengths used for Cu (324.8 nm), Cr (360.5 nm), Mn (279.5 nm), and Pb (283.3 nm), respectively. The analyte signal acquisition mode was obtained by area peak in agreement to the concentration level of the analyte.

Reagents and Solutions

Prior to analysis, all glass and plastic recipients were immersed in a 10% (v v^{-1}) HNO_3 solution for 24 h followed by rinsing with ultrapure water (resistivity higher than 18.2 $\text{M}\Omega\text{ cm}$). All solutions were prepared with HNO_3 previously distilled in a quartz sub-boiling system (Distillacid, Berghof). The aqueous standard solutions employed were prepared using 1000 mg L^{-1} of each element Cu, Cr, Mn and Pb (SpecSol) in 1% (v v^{-1}) HNO_3 solution. Stock solutions of each chemical modifier used, $\text{Pd}(\text{NO}_3)_2$ and $\text{Mg}(\text{NO}_3)_2$, were prepared by dissolving 0.6259 g of

Pd(NO₃)₂·2H₂O (Sigma-Aldrich), and 2.6709 g of Mg(NO₃)₂·6H₂O (Sigma-Aldrich,) in a 25 mL 1% (v v⁻¹) HNO₃ solution.

The ALF composition is the closest to human pulmonary environments, simulating the acidic cellular conditions under the phagocytosis process and it was prepared as described by Colombo et al.¹⁸ (Table S1).

In the experiments of the GFAAS instrumental conditions optimization, the recovery parameter was evaluated, being obtained by the ratio of the signal from ALF samples previously spiked with the analyte and the signal from the standard analyte solution (reference solution), both in the same concentration level. In these experiments, the final volume of ALF employed was about 5 mL.

Instrumental Parameters

The general instrumental conditions that were optimized for the analysis of the four elements by GFAAS are show in Table I. For that purpose spiked ALF samples and standard solutions were used.

Table I. General heating program for the direct determination of Cu, Cr, Mn and Pb in ALF samples by GFAAS.

Step	Temperature (° C)	Ramp Time (s)	Hold Time (s)	Gas flow rate (L min⁻¹)
Drying 1	85	5	0	0.1
Drying 2	110	50	0	0.1
Pyrolysis 1	T _{pyr}	10	10	1
Pyrolysis 2	T _{pyr}	0	3	0
Atomization	T _{atom}	0	3	0
Clean	2500	0	2	1

Determination of Cu, Cr, Mn and Pb in the PM_{2.5} ALF-soluble fraction

PM_{2.5} aerosol samples were collected on 37mm polycarbonate filters (Nuclepore) using a Harvard impactor (Air Diagnostics, Harrison, ME, USA) with flow rate of 10 L min⁻¹, at 2 m from the ground.²⁸ The pilot study was located at the National Institute of Meteorology (INMET) station in Curitiba city, Paraná State, Brazil, (coordinates 25°26'93'' S, 49°13'85'' O). The sampling site is a residential area near the city center, adjacent to two important highways with a significant flow of light and heavy duty vehicles.

To mimic the solubilization process of aerosol in the human lung, PM_{2.5} collected on filters were subjected to an *in-vitro* test.¹⁸ To this end, to each entire collected filter 6.00 mL of ALF was added. The samples were then incubated at 37 °C and agitated at 40 cycles per minute for 1 hour and filtered using polyvinylidene difluoride (PVDF) membrane filters (0.22 μm pore size, Vertipure) to separate the supernatant (bioaccessible fraction) from the insoluble fraction. The supernatant was directly analyzed by GFAAS. A method blank was prepared in the same manner.

Concentrations are reported as μg m⁻³ to enable comparison with air pollution guideline values.

Results and Discussion

Instrumental Conditions study for the Cu, Cr, Mn and Pb in an ALF matrix

To optimize the pyrolysis and atomization temperatures, the ALF was spiked with 25 μg L⁻¹ Cu in the presence of a suitable chemical modifier. In general, a univariate study is performed in order to achieve the optimal pyrolysis temperature (T_{pyr}) and atomization temperature (T_{atom}).

In Fig. 1, the pyrolysis curve shows that above 1200 °C the absorbance signal decreased indicating a Cu loss, possibly due to its volatilization. Therefore, the optimal T_{pyr} of 1100 °C was chosen taking into account the suitable thermal conditions providing the elimination of most of the

matrix interferences to a fixed T_{atom} of 2300 °C. Then, the optimal T_{pyr} (1100 °C) was maintained to investigate the influence of varying T_{atom} (range from 1900 °C to 2400 °C). As can be observed in Fig. 1, the T_{atom} of 2100 °C can be considered suitable, taking into account the satisfactory sensitivity (indicated by signal-to-background-ratio (SBR)) as observed by the Cu signal. In all experiments, the ratio of Cu absorbance signal from ALF matrix and the Cu absorbance in 1% (v v⁻¹) HNO₃ (reference solution), both at the same concentration level, was evaluated in order to verify the optimal thermal analysis conditions. In all experiments the chemical modifier solution containing 5 µg Pd : 0.5 µg Mg was employed. The range of the recovery values obtained for Cu 87-101%.

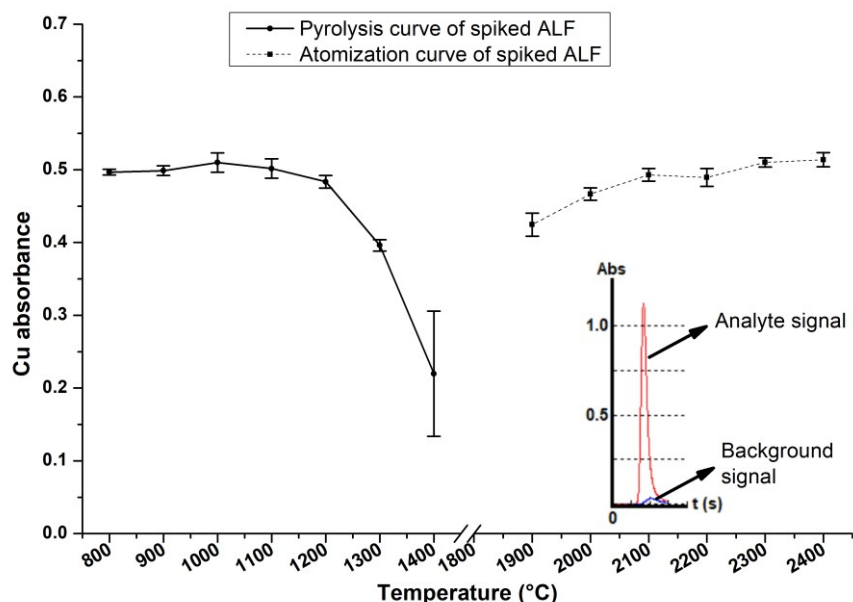


Figure 1. Pyrolysis and atomization temperatures optimized for ALF samples spiked with 25 µg L⁻¹ copper. Sample volume: 20 µL, chemical modifier: 5 µL. The optimization experiments were performed in triplicate, n=3.

Chromium analyses with AAS methods are notoriously difficult due to the refractory nature of Cr which requires high atomization temperatures. In addition, Cr determination is highly wavelength dependent. For that reason the method was optimized by systematically studying the variation in the ratio of chromium absorbance signal of the ALF and 1% (v v⁻¹) HNO₃ solutions

spiked with $25 \mu\text{g L}^{-1}$ Cr with variation in wavelength. The optimized wavelength was found to be 360.5 nm, using $2 \mu\text{g}$ of magnesium as chemical modifier.

The optimal T_{pyr} of $1550 \text{ }^\circ\text{C}$ was chosen (Fig. 2) because it produced the lowest SBR and the best recovery values for the Cr in different media. The atomization temperature of $2400 \text{ }^\circ\text{C}$ produced the highest absorbance signal for chromium in ALF matrix. Using these optimal conditions recovery values of 93% were obtained which can be considered acceptable quantification in the presence of the matrix interferences.²⁹⁻³¹

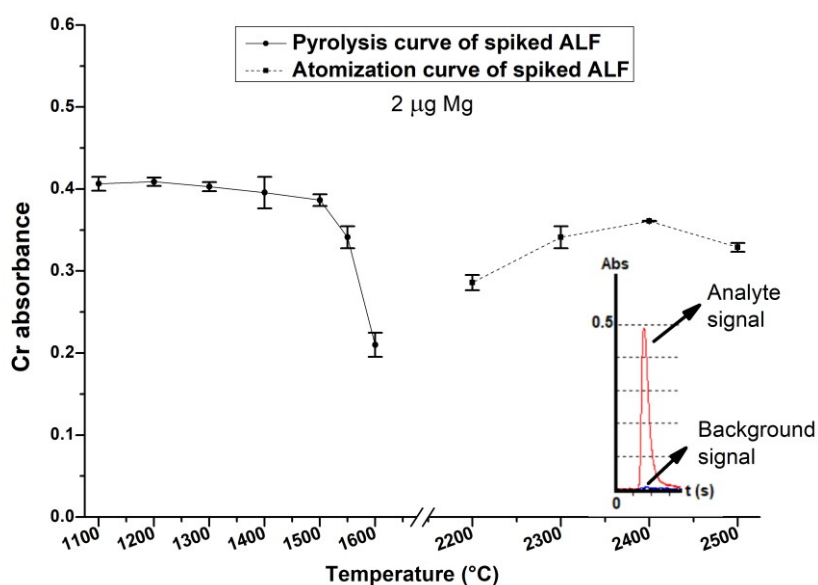


Figure 2. Pyrolysis and atomization temperatures optimization for ALF samples spiked with $25 \mu\text{g L}^{-1}$

chromium. Sample volume: $20 \mu\text{L}$, chemical modifier: $5 \mu\text{L}$. The optimization experiments were performed in triplicate, $n=3$.

For the Mn quantification $2 \mu\text{g Mg}$ was used as chemical modifier. For the fixed T_{atom} of $2200 \text{ }^\circ\text{C}$, an optimal T_{pyr} of $1200 \text{ }^\circ\text{C}$ was chosen as can be observed in Fig. 3. The Mn recovery values were 88 – 105 %. The T_{atom} previously defined ($T_{\text{a}} = 2200 \text{ }^\circ\text{C}$) providing suitable conditions for the Mn quantification without further optimization. This is most likely due to a T_{pyr} promoting suitable reaction conditions that improved the volatility.

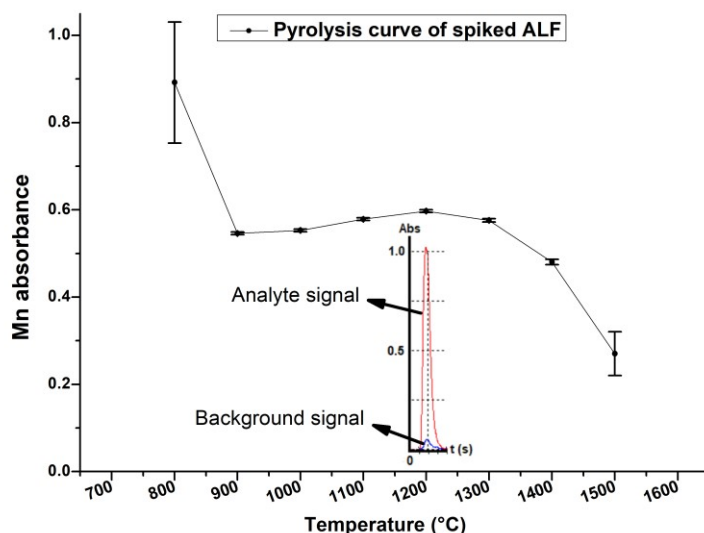


Figure 3. Pyrolysis temperature optimization at T_{atom} fixed at 2200 °C for ALF samples spiked with 25 $\mu\text{g L}^{-1}$ manganese. Sample volume: 20 μL , chemical modifier: 5 μL . The optimization experiments were performed in triplicate, $n=3$.

For the Pb quantification, the matrix interferences on the Pb signal could only be solved employing the factorial design as the Central Composite Design (CCD). The pyrolysis and atomization temperatures in GFAAS are generally optimized using univariate methods (one-at-a-time variation in parameter) in which case the interaction effects between the studied variables are not generally evaluated.³² The use of the factorial design is based a multivariate optimization strategy that allows the identification of the factor, as well as the possible interactions that may affect the result of an experiment. In this study, the response variable (recovery values) was measured for all possible combinations of the chosen pyrolysis and atomization temperature levels. For the Pb quantification, the pyrolysis and atomization temperatures were evaluated by Central Composite Design (CCD), which presents 5 levels (-2, -1, 0, +1 and 2 square root), as presented in Table S2. This approach allowed successful optimization of Pb determination in this matrix, which could not be achieved using the univariate approach followed for Cu, Cr, and Mn.

The recovery was also evaluated by ratio from absorbance signal between Pb spiked ALF and Pb standard solutions (reference solution) both at the same concentration level, 50 $\mu\text{g L}^{-1}$ Pb. In these experiments a chemical modifier containing 5 $\mu\text{g Pd}$: 3 $\mu\text{g Mg}$ was used. The Fig. 4 shows the

profile of the response surface under each instrumental condition evaluated. These experiments were performed randomly, and the statistical procedures were done using Statistica 7.0 software (StarSoft, Tulsa, USA).

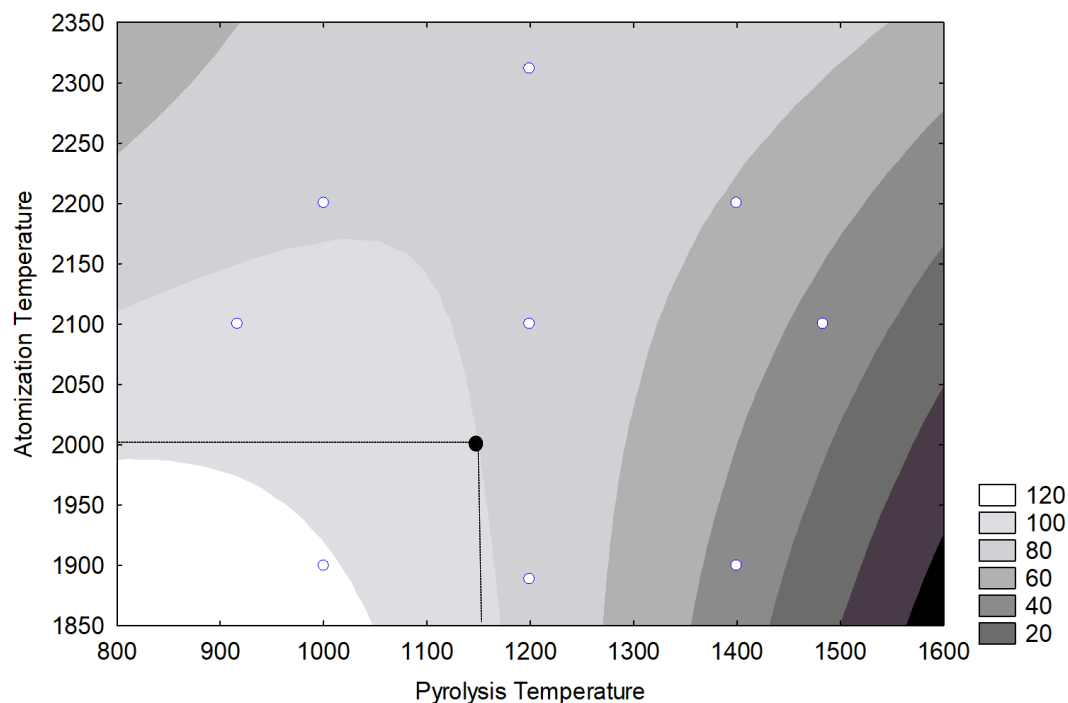


Figure 4. Contour diagram obtained for the ratio of absorbance values between Pb spiked ALF samples and Pb standard solution, both containing $50 \mu\text{g L}^{-1}$ Pb in the presence of chemical modifier ($5 \mu\text{g Pd} + 3 \mu\text{g Mg}$). Sample volume: $20 \mu\text{L}$, chemical modifier: $5 \mu\text{L}$. The optimization experiments were performed in duplicate, $n=2$.

In this study, the recovery value (response variable) depends on the possible interactions between the response variable as well as the levels of two variables (T_{pyr} and T_{atom}) studied by the CCD design that can be represented by a contour diagram Fig. 4.

Satisfactory recovery values (80-100%) for Pb in ALF were obtained applying a T_{pyr} of $1150 \text{ }^\circ\text{C}$ and a T_{atom} of $2000 \text{ }^\circ\text{C}$, as indicated in Fig.4. The recovery value on each contour was constant, and the target optimum instrumental condition is indicated. The accuracy of the developed methods was evaluated by addition-recovery experiments in the ALF, in agreement with the aqueous calibration curve (Table II).

Table II. Addition-recovery values obtained for Cu, Cr, Mn and Pb in ALF by GFAAS (mean value \pm sd, n=3).

	Concentration level added ($\mu\text{g L}^{-1}$)*	Found Value ($\mu\text{g L}^{-1}$)	Recovery (%)
Cu	2	2 ± 0.1	100
	10	11 ± 0.1	110
	20	18 ± 1	90
	30	27 ± 0.4	90
	50	52 ± 0.3	104
	80	78 ± 3	97
Cr	4	5 ± 0.3	125
	10	11 ± 0.4	110
	25	25 ± 1	100
Mn	2	2 ± 0.2	100
	10	10 ± 0.1	100
	15	15 ± 0.1	100
	25	23 ± 0.2	93
	35	34 ± 0.5	98
Pb	10	8 ± 0.1	80
	20	22 ± 0.1	110
	50	49 ± 0.5	98
	70	68 ± 0.3	97
	100	93 ± 2	93

* different amounts of the standard solution were added into the fix sample volume (5 mL) of ALF for each concentration level evaluated.

Satisfactory recovery values were obtained and different calibration curves for Cu, Mn and Pb were used due to varying in the concentration levels of the PM_{2.5} samples collected in the city of Curitiba.¹⁶ The analytical performance parameters of the method were evaluated, as required (Table S3).³³

The ALF samples containing Cu, Cr, Mn and Pb were analyzed by GFAAS and the results are shown in Table III.

Table III. Soluble concentration ($\mu\text{g L}^{-1}$) in ALF and atmospheric soluble concentration (ng m^{-3}) in $\text{PM}_{2.5}$ for Cu, Cr, Mn and Pb in the ALF by GFAAS, (mean value \pm sd, n=3).

Sample	Air Volume	Soluble Concentration in ALF ($\mu\text{g L}^{-1}$)				Atmospheric soluble concentration (ng m^{-3})			
	(m^3)	Cu	Cr	Mn	Pb	Cu	Cr	Mn	Pb
1	216	35 ± 0.01	5.1 ± 0.41	17 ± 2	71 ± 1	0.96 ± 0.0002	0.14 ± 0.01	0.47 ± 0.04	1.9 ± 0.04
2	103	20 ± 0.1		13 ± 0.1	144 ± 1	1.1 ± 0.01		0.75 ± 0.01	8.4 ± 0.08
3	99	16 ± 0.4	5.0 ± 0.01	8.3 ± 0.20	66 ± 1	0.98 ± 0.02	0.30 ± 0.001	0.50 ± 0.01	4.0 ± 0.08
4	100	20 ± 0.04		11 ± 0.1	124 ± 0.4	1.2 ± 0.003		0.64 ± 0.01	7.4 ± 0.02
5	102	26 ± 0.4		12 ± 0.04	24 ± 0.5	1.6 ± 0.02		0.68 ± 0.003	1.4 ± 0.03
6	99	14 ± 0.2	4.1 ± 0.11	10 ± 0.03	24 ± 0.1	0.86 ± 0.01	0.25 ± 0.01	0.59 ± 0.002	1.5 ± 0.01
7	100	39 ± 0.6	12 ± 0.3	13 ± 0.2	136 ± 1	2.4 ± 0.03	0.71 ± 0.02	0.79 ± 0.01	8.2 ± 0.07
8	115	16 ± 0.02	7.4 ± 0.72	11 ± 0.07	113 ± 3	0.83 ± 0.001	0.39 ± 0.04	0.57 ± 0.004	5.9 ± 0.17
Average						1.2 ± 0.01	0.41 ± 0.01	0.62 ± 0.01	4.8 ± 0.03

The results of the soluble concentration of each chemical element investigated in the ALF were calculated as atmospheric concentration (Table III). The mean atmospheric soluble concentrations of the analytes in the samples were $1.2 \pm 0.01 \text{ ng m}^{-3}$ Cu, $0.41 \pm 0.01 \text{ ng m}^{-3}$ Cr, $0.62 \pm 0.01 \text{ ng m}^{-3}$ Mn, and $4.8 \pm 0.03 \text{ ng m}^{-3}$ Pb.

Considering the historical elemental average of $\text{PM}_{2.5}$ in Curitiba as being Cu 3.3 ng m^{-3} , Cr 2.1 ng m^{-3} , Mn 6.1 ng m^{-3} , and Pb 21 ng m^{-3} ,¹⁶ the bioaccessibility percentage of the analyzed samples were calculated to be Cu 38%; Cr 20%; Mn 10%; and Pb 23% (where bioaccessibility is defined as the ratio between the mass leached onto ALF and the total mass).

While the evidence for differential solubility of particulate matter is far from conclusive, Wiseman and Zereini²⁰ evaluated the soluble fraction of some chemical elements in ALF simulated lung fluid using a similar *in vitro* procedure but a difference in incubation time (24 hours in comparison to 1 hour in this investigation). They found that the soluble inorganic content of the samples was: 42.4 ng m^{-3} Cu, 3.0 ng m^{-3} Cr, 9.9 ng m^{-3} Mn and 10.9 ng m^{-3} Pb, indicating higher solubility level of $\text{PM}_{2.5}$ in comparison with the city of Curitiba, Brazil. However, considering the difference in incubation period, the results for Cr, Mn and Pb seem to be reasonably close to each other.

The values indicated obtained for the Cu, Cr and Mn in the atmospheric soluble fraction were lower than the inhalation risk concentrations as reported: Cu $100 \mu\text{g m}^{-3}$,¹¹ Cr 8 ng m^{-3} ,¹² and Mn 50 ng m^{-3} .¹³ However, for Pb, the atmospheric soluble fraction of all samples analyzed exceeded the inhalation risk unit of 0.012 ng m^{-3} .¹⁴ The chemical health risk assessment analysis of Pb exposure was performed with the average soluble concentration of Pb in ALF and the inhalation risk unit based on the previously methods developed by the United States Environmental Protection Agency using the Risk Assessment Information System.³⁴ This method modeled the carcinogenic risks that may occur due to Pb exposure through inhalation of the $\text{PM}_{2.5}$. The results indicate the probability of 2 residents develop carcinogenic diseases over a lifetime in each group of 100 million inhabitants.

Currently, Pb is not an additive used in Brazilian gasoline, but it can be found as a trace contaminant in diesel generating significant emissions of Pb to the atmosphere by combustion of these fuels, e.g., old trucks, locomotives, and thermal power plants.²¹ The values reported here stresses that it is plausible to assume that long-term low-dose exposure will have a severe effect on public health in the city.

Conclusions

We have shown that GFAAS with Deuterium lamp correction capability can be used to quantify Cu, Cr, Mn, and Pb in a matrix with high salt concentration. The developed method provided suitable sensitivity and accuracy despite the absence of Zeeman correction capability. Furthermore, minimal sample preparation was required and pre-treatment was limited to relative simple chemical modifiers that is inexpensive. The optimized conditions was used to determine the concentration of Cu, Cr, Mn, and Pb from leachates produced after PM_{2.5} samples were incubated at 37 °C in an ALF with high ionic strength. Results showed comparable values to those published in literature. This robust and straightforward GFAAS method is pivotal for low and middle-income countries where most air pollution adverse effects occur, and more sophisticated technologies are not always available.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgment

Financial support of Coordination for the Improvement of Higher Education Personnel (CAPES), the National Council for Scientific and Technological Development (CNPq), and the

Araucaria Foundation for Scientific and Technological Development of Parana (Grant no. 435/2014)

ORCID ID

Andrea Oliveira (<https://orcid.org/0000-0003-4381-9908>)

Supplemental Material

The supplemental material mentioned in the text, consisting of the important parameters that support the data obtained in this work and it is available in the online version of the journal.

References

1. C.A. Pope, M. Ezzati, D.W. Dockery. "Fine-Particulate Air Pollution and Life Expectancy in the United States". *N. Engl. J. Med.* 2009. 360: 376-386.
2. C. Canova, C. Dunster, F.J. Kelly, C. Minelli, P.L. Shah, C. Caneja, M.K. Tumilty, P. Burney. "PM10-Induced Hospital Admissions for Asthma and Chronic Obstructive Pulmonary Disease the Modifying Effect of Individual Characteristics". *Epidemiol.* 2012. 23: 607-615.
3. C. Angelé-Martínez, C. Goodman, J. Brumaghim. "Metal-Mediated DNA Damage and Cell Death: Mechanisms, Detection Methods, and Cellular Consequences". *Metallomics.* 2014. 6(8): 1358-1381.
4. S. Becker, A.D. Lisa, M.S. Joleen, C.G. teven, B.D. Robert, T.H. Yuh-Chin. "Seasonal Variations in Air Pollution Particle-Induced Inflammatory Mediator Release and Oxidative Stress". *Environ. Health Persp.* 2005. 113(8): 1032-1038.
5. P. Chen, S. Chakraborty, S. Mukhopadhyay, E. Lee, M.M.B. Paoliello, A.B. Bowman, M. Aschner. "Manganese Homeostasis in the Nervous System". *J. Neurochem.* 2015. 134(4): 601-610.

6. G.C. Fang, Y.C. Chen, C.T. Lo, M.H. Cho, Y.J. Zhuang, K.H. Tsai, C.Y. Huang, Y.F. Xiao. "Concentrations and Analysis of Health Risks of Ambient Air Metallic Elements at Longjing Site in Central Taiwan". *Environ. Geochem. Health*. 2018. 40(1): 461-472.
7. R.H.M. Godoi, G. Polezer, G.C. Borillo, A. Brown, F.B. Valebona, T.O.B. Silva, A.B. Ingberman, M. Nalin, C.I. Yamamoto, S. Potgieter-Vermaak, R.A. Penteado Neto, M.R. de Marchi, P.H. Saldiva, T. Pauliqueves, A.F. Godoi "Influence on the Oxidative Potential of a Heavy-Duty Engine Particle Emission due to Selective Catalytic Reduction System and Biodiesel Blend". *Sci. Total Environ*. 2016. 560: 179-185.
8. P.B. Kurt-Karakus. "Determination of Heavy Metals in Indoor Dust from Istanbul, Turkey: Estimation of the Health Risk". *Environ. Int*. 2012. 50: 47-55.
9. J.A. Fisher, R.C. Puett, F. Laden, G.A. Wellenius, A. Sapkota, D. Liao, et al. "Case-crossover analysis of short-term particulate matter exposures and stroke in the health professionals follow-up study". *Environ Int*. 2019. 124: 153-160.
10. C.I. Davidson, R.F. Phalen, P.A. Solomon. "Airborne Particulate Matter and Human Health: A Review". *Aerosol Sci. Technol*. 2005. 39: 737-749.
11. US EPA, Hazardous Air Pollutants, Health Effects Notebook for Hazardous Air Pollutants, 2017. Available: <http://www.epa.gov.br> [accessed Nov 12 2019]
12. IRIS. Integrated Risk Information System. U.S. Environmental Protection Agency Chemical Assessment Summary, Chromium (VI) (CASRN 18540-29-9), 2003. Available: <https://cfpub.epa.gov> [accessed Oct 21 2019]

13. IRIS. Integrated Risk Information System. U.S. Environmental Protection Agency Chemical Assessment Summary, Manganese, (CASRN 7439-96-5) 2002. Available: <https://cfpub.epa.gov> [accessed Oct 21 2019]
14. Cal/EPA. The California Environmental Protection Agency. Toxic Air Contaminant List with Staff Reports/Executive Summaries, Inorganic Lead TAC - Full Document, 1997.
15. S. Potgieter-Vermaak, G. Rotondo, V. Novakovic, S. Rollins, R. Van Grieken. "Component-Specific Toxic Concerns of the Inhalable Fraction of Urban Road Dust". *Environ. Geochem. Health* 2012. 34: 689-696.
16. G. Polezer, Y.S. Tadano, H.V. Siqueira, A.F.L. Godoi, C.I. Yamamoto, P.A. de André, T. Pauliquevis, M.F. Andrade, A. Oliveira, P.H.N. Saldiva, P.E. Taylor, R.H.M. Godoi. "Assessing the Impact of PM_{2.5} on Respiratory Disease using Artificial Neural Networks". *Environ. Pollut.* 2018. 235: 394-403.
17. N.B. Ivanenko, A.A. Ganeev, N.D. Solovyev, L.N. Moskvina. "Determination of Trace Elements in Biological Fluids". *J. Anal. Chem.* 2011. 66: 784-799.
18. C. Colombo, A.J. Monhemius, J.A. Plant. "Platinum, Palladium and Rhodium Release from Vehicle Exhaust Catalysts and Road Dust Exposed to Simulated Lung Fluids". *Ecotoxicol. Environ. Saf.* 2008. 71(3): 722-730.
19. C. Julien, P. Esperanza, M. Bruno, L.Y. Alleman. "Development of an in Vitro Method to Estimate Lung Bioaccessibility of Metals from Atmospheric Particles". *J. Environ. Monitor.* 2011. 13(3): 621-630.

20. C.L.S. Wiseman, F. Zereini. "Characterizing Metal(loid) Solubility in Airborne PM10, PM2.5 and PM1 in Frankfurt, Germany Using Simulated Lung Fluids". *Atmos. Environ.* 2014. 89: 282-289.
21. S.W. Li, H.B. Li, J. Luo, H.M. Li, X., Qian X, M.M. Liu, J. Bi, X.Y. Cui, L.Q. Ma. "Influence of Pollution Control on Lead Inhalation Bioaccessibility in PM2.5: A Case Study of 2014 Youth Olympic Games in Nanjing". *Environ. Int.* 2016. 94: 69-75
22. P. Coufalík, P. Mikuska, T. Matousek, Z. Vecera. "Determination of the Bioaccessible Fraction of Metals in Urban Aerosol using Simulated Lung Fluids". *Atmos. Environ.* 2016. 140: 469-475.
23. W.E. Hillwalker, K.A. Anderson. "Bioaccessibility of Metals in Alloys: Evaluation of Three Surrogate Biofluids". *Environ. Pollut.* 2014. 185: 52-58.
24. A. Mukhtar, V. Mohr, A. Limbeck. "The Suitability of Extraction Solutions to Assess Bioaccessible Trace Metal Fractions in Airborne Particulate Matter: A Comparison of Common Leaching Agents". *Environ. Sci. Pollut. Res. Int.* 2015. 22(21): 16620-16630.
25. Z-J. Tang, X. Hu, J.-Q. Qiao, H.-Z. Lian. "Size Distribution, Bioaccessibility and Health Risks of Indoor/Outdoor Airborne Toxic Elements Collected from School Office Room". *Atmosphere.* 2018. 9(9): 340-353.
26. W. Stopford, J. Turner, D. Cappellini, T. Brock. "Bioaccessibility Testing of Cobalt Compounds". *J. Environ. Monit.* 2003. 5(4): 675-680.
27. K. Midander, J. Pan, I.O. Wallinder, C. Leygraf. "Metal Release from Stainless Steel Particles in Vitro-Influence of Particle Size". *J. Environ. Monit.* 2007. 9(1): 74-81.
28. V.A. Marple, K.L. Rubow, W. Turner, J.D. Spengler. "Low Flow Rate Sharp Cut Impactors for Indoor Air Sampling: Design and Calibration" *JAPCA.* 1987. 37: 1303-1307.

29. C.E.R. Paula, G.F.B. Cruz, C.M.S.P. Rezende, R.J. Cassella. "Determination of Cr and Mn in Moisturizing Creams by Graphite Furnace Atomic Absorption Spectrometry through Direct Introduction of the Samples in the Form of Emulsions". *Microchem. J.* 2016. 127: 1-6.

30. R. Dobrowolski. "Determination of Ni and Cr in Soils by Slurry Graphite Furnace Atomic Absorption Spectrometry". *Spectrochim. Acta B* 1996. 51(2): 221-227.

31. M. Wójciak-Kosior, W. Szwerc, M. Strzemiński, Z. Wichłacz, J. Sawicki, R. Kocjan, M. Latański, I. Sowaa. "Optimization of High-Resolution Continuum Source Graphite Furnace Atomic Absorption Spectrometry for Direct Analysis of Selected Trace Elements in Whole Blood Samples". *Talanta*. 2017. 165: 351-356.

32. J.N. Miller, J.C. Miller. "Statistics and Chemometrics for Analytical Chemistry". England: Pearson Education Ltd., 2005. Fifth ed.

33. European communities. Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Communities, (OJEC)* (2002) 3044.

34. R.H.M. Godoi, A.F.L. Godoi, L.C. de Quadros, G. Polezer, T.O.B. Silva, C.I. Yamamoto, R. van Grieken, S. Potgieter-Vermaak. "Risk Assessment and Spatial Chemical Variability of PM Collected at Selected Bus Stations". *Air Qual. Atmos. Health* 2013 6: 725-735.

Figure Captions

Figure 1. Pyrolysis and atomization temperatures optimized for ALF samples spiked with $25 \mu\text{g L}^{-1}$ copper. Sample volume: $20 \mu\text{L}$, chemical modifier: $5 \mu\text{L}$. The optimization experiments were performed in triplicate, $n=3$.

Figure 2. Pyrolysis and atomization temperatures optimization for ALF samples spiked with $25 \mu\text{g L}^{-1}$ chromium. Sample volume: $20 \mu\text{L}$, chemical modifier: $5 \mu\text{L}$. The optimization experiments were performed in triplicate, $n=3$.

Figure 3. Pyrolysis temperature optimization at T_{atom} fixed at $2200 \text{ }^{\circ}\text{C}$ for ALF samples spiked with $25 \mu\text{g L}^{-1}$ manganese. Sample volume: $20 \mu\text{L}$, chemical modifier: $5 \mu\text{L}$. The optimization experiments were performed in triplicate, $n=3$.

Figure 4. Contour diagram obtained for the ratio of absorbance values between Pb spiked ALF samples and Pb standard solution, both containing $50 \mu\text{g L}^{-1}$ Pb in the presence of chemical modifier ($5 \mu\text{g Pd} + 3 \mu\text{g Mg}$). Sample volume: $20 \mu\text{L}$, chemical modifier: $5 \mu\text{L}$. The optimization experiments were performed in duplicate, $n=2$.