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Lead biomonitoring in different organs of lead intoxicated rats employing GF AAS and different sample preparations

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ABSTRACT

An analytical procedure was developed for the determination of lead in different tissues from Wistar Hanover rats, previously intoxicated with lead acetate during a toxicological study. About 25 mg of dried sample (bone, liver, kidney, heart, lung and spleen) were mixed with 8.0 mL of 7.00 mol L^{-1} nitric acid and digested using microwave radiation in closed vessel. Except for the bone samples, the other tissues could also be analyzed after alkaline solubilization with TMAH. All the digested or solubilized samples were analyzed by graphite furnace atomic absorption spectrometry. Good accuracy and precision were attained when analyzing reference standard materials (for bone, liver and kidney) and also from addition to recovery experiments (for heart, lung and spleen tissues). The method was applied to samples from nine animals and the results suggested that there is a profile for lead bioaccumulation in these animals, which seemed to adapt themselves to continuous lead exposure.

1. Introduction

Due to historical [1] and toxicological aspects [2] lead is one of the more important metallic species. For living beings, mainly mammals, there is no evidence of essential function for this element, which trends to accumulate in the bones and causes many biochemical alterations [2–4].

Such alterations are considered irreversible and have the following symptons for the human beings: anemia, muscular fatigue, cognitive problems in children, hyperactivity, irritation, gastrointestinal problems, diminished fertility (for males) and reduced renal functions [2].

Consequently, it is current practice to treat individuals contaminated with lead in order to reduce their levels of contamination, as is done at the Clinical Hospital of the University of Campinas (UNICAMP). In general, these patients are individuals with bullets inside their bodies [5] as well as professionals that have direct or indirect contact with lead such as shooting instructors [6] and the employees of foundries [7]. On the other hand, the amount of lead in the various affected organs has not being considered in toxicological studies, or at least, has not been reported.

The effects caused by lead intoxication and the fact that this element tends to accumulate in the bones are well known issues. However, how the organs are involved in the bioaccumulation process and the extent of its bioaccumulation is not yet known.

In this context, the objective of this work is to present an analytical protocol (and its application) specifically designed for the determination of lead in bones and different organs (liver, kidney, heart, lung and spleen) of Wistar Hanover rats, which are generally the animals used in this kind of experimental study.

Regarding the sample preparation possibilities, there are three ordinary procedures to decompose the organic sample matrix of those biological tissues in its inorganic constituents: dry ashing, hot plate digestion or microwave digestion, which may be carried out prior to the spectrometric analysis and commonly in acid media. However, microwave digestion methods are preferred because they are faster, more precise, avoid the loss of volatile elements besides using small amounts of reagents [8].

On the other hand, alkaline solubilization of biological materials was also shown to be an alternative for acid digestion in the cases of some speciation analysis and when small sample amounts are available. In this latter, the whole sample treatment can be done in a single vessel and the use of small dilution volumes is then facilitated. For this purpose TMAH® is one of the most used reagents, mainly before analysis by GF AAS or techniques using an inductively coupled plasma source (ICP OES or ICP-MS) [9].

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Considering the information above the determination of lead, in this work, was carried out by Graphite Furnace Atomic Absorption Spectrometry (GF AAS) after different sample preparations (acid digestion in a microwave oven or alkaline solubilization with ammonium tetramethylhydroxide—TMAH) in biological tissues. The proposed method was shown to be appropriate for this kind of sample and all the selected tissues could be analyzed due to the good GF AAS detectability (detection limits at the $\mu g\ kg^{-1}$ level [10]), precision and small sample requirements.

2. Experimental

2.1. Equipments and reagents

All chemical reagents were of analytical grade. Deionized water (resistivity of 18.2 MΩ cm) was obtained with a Milli-Q System (Millipore, Bedford, MA, USA). Nitric acid (Merck, Darmstadt) was distilled before use. All sample containers, autosampler cups and other materials were washed with neutral soap, soaked in 10% (v/v) nitric acid for 24 h and rinsed with deionized water prior to use.

A 1000 $\mu g\ mL^{-1}$ lead solution (J. T. Baker, Center Valley, PA, USA) was used to prepare the analytical standards in 0.2% (v/v) HNO_3, with concentrations between 1.0 and 50 $\mu g\ L^{-1}$. The chemical modifier was prepared from solid $NH_4H_2PO_4$ (Ecibra, São Paulo), solid $Mg(NO_3)_2$ (Merck) and solid $Pd(NO_3)_2$ (Merck).

A microwave oven from Milestone (model ETHOS 1) was used for acid sample digestion employing nitric acid, while for alkaline sample solubilization 25% (v/v) tetramethylammonium hydroxide, TMAH (Fluka, St. Louis, USA) was used without heating.

All analytical measurements were made in a graphite furnace atomic absorption spectrometer from Perkin-Elmer (Norwalk, CT, USA, model A Analyst 600). This spectrometer is equipped with a longitudinal Zeeman background corrector, auto sampler (model AS-800), standard graphite tube with integrated Lvov platform and transversal heating (THGA). The radiation source was a Pb electrodeless discharge lamp—EDL ($\lambda = 283.3$ nm; $i = 360$ mA) with slit widths of 0.7 nm or 0.2 nm (for bone analysis). Pure argon was employed as the inert gas at a flow rate of 250 $mL\ min^{-1}$. All the measurements were made in integrated absorbance units.

Table 1
Microwave digestion program employed for acid digestion of the bones of Wistar Hanover rats.

Step	Time (min)	Temperature (°C)
1-Ramp	6	80
2-Hold time	2	80
3-Ramp	3	120
4-Hold time	4	120
5-Ramp	10	180
6-Hold time	15	180
7-Exhaust	40	0

2.2. Animals intoxication and sample treatment

The animals studied were male rats (Wistar Hanover) that were raised under the same condition (environment and feed availability) at the *Experimental Medicine Department* of the Unicamp Medical School, following the *Brazilian College for Animal Experimentation* authorization (Protocol 1600-1—Adaptative processes in Wistar rats during subacute lead intoxication) and rigorous ethical principles.

In order to intoxicate the animals, an aliquot of 0.50 mL of 1% (m/m) lead acetate in distilled water was daily administrated to each test animal, while for the control animals, which were not intoxicated, lead acetate was substituted by daily aliquots of 0.50 mL of distilled water (placebo). In both cases the animals were treated for different periods of time (eight, fifteen, thirty and sixty days) by the administration of the lead solution or the placebo through intra-peritoneal injections. At the end of each testing periods, the animals were sacrificed, and following this, the organs (liver, kidney, heart, lung and spleen) and femur were extracted and dried at 70 °C for 72 h. Afterwards, the bone tissue was milled with a knife mill (IKA, A11 Basic) and then digested in the microwave oven. The other tissues were solubilized in alkaline media, due to the small amounts obtained of these dried samples. Finally, the treated samples (organs and bone) were diluted to 25 mL with deionized water and analyzed under the selected conditions.

2.2.1. Acid digestion for bone and organs

This procedure was studied for all sample tissues while developing the method but for the biomonitoring study this digestion was only used for the bone samples. About 0.25 g of each dried tissue were mixed with 8.0 mL of 7.00 mol L^{-1} HNO_3 acid in PTFE vessels and left in predigestion for 30 min. Then, the vessels were closed and the samples were digested employing the microwave oven operating under the conditions shown in Table 1.

2.2.2. Alkaline solubilization

The alkaline solubilization was very useful to deal with small sample amounts, mainly for the spleen and heart, and all the preparation procedure was made in the same flask following a sample/reagent proportion optimized for 25 mg of sample. In this case, 1.00 mL of 25% (v/v) TMAH was added to the dried tissue and the mixture was left overnight at room temperature. Then the solubilized tissue was diluted to 25 mL with deionized water and analyzed after manual agitation.

2.3. Instrument optimization

2.3.1. Conditions for the analysis of acid digests

In order to optimize the instrumental conditions, pyrolysis and atomization curves were built for each tissue, previously fortified with lead and after digesting according to Section 2.2.1. Compromise

Table 2
Instrumental parameters used to determine Pb^{2+} in the tissues of Wistar Hanover rats.

Instrumental parameter	Level for acid digested samples	Level for solubilized samples (TMAH)
Wavelength (nm)	283.3	283.3
Slit width (nm)	0.2 (bone) and 0.7 (other tissues)	0.7
Chemical modifier	50 μg $NH_4H_2PO_4$ + 3 μg $Mg(NO_3)_2$	5 μg Pd + 3 μg $Mg(NO_3)_2$
Pyrolysis temperature	850 °C	950 °C
Atomization temperature	1550 °C	1700 °C
Replicates	2	2
Analytical curve media	0.2% (v/v) HNO_3	1.0% (v/v) TMAH
Calibration type	Linear with calculated intercept	Linear with calculated intercept

Table 3
Heating program used to determine Pb^{2+} in digested rat tissues.

Step	Temperature (°C)	Ramp (s)	Hold time (s)	Ar flow rate (mL min)$^{-1}$
Drying	110; 130	7; 15	30; 30	250
Pyrolysis	850	10	20	250
Atomization	1550	0	3	0
Cleaning	2450	1	3	250

Table 4
Heating program used to determine Pb^{2+} in solubilized rat tissues.

Step	Temperature (°C)	Ramp (s)	Hold time (s)	Ar flow rate (mL min)$^{-1}$
Drying	110; 130	7; 15	30; 30	250
Pyrolysis	950	10	20	250
Atomization	1700	0	3	0
Cleaning	2450	1	3	250

conditions were selected by comparing the integrated absorbances for the different digests and are shown in Tables 2 and 3. Accuracy and precision tests were also done by analyzing certified reference materials for bone, liver and kidney, while for heart, lung and spleen tissues addition and recovery experiments were carried out.

2.3.2. Conditions for the analysis of alkaline samples

As for the acid digestion the main instrumental parameters (pyrolysis and atomization temperatures) were evaluated also for the solubilized tissues. The selected compromise conditions are shown in Tables 2 and 4 and the accuracy was validated as the same way done for the digested tissues.

2.4. Sample analysis for Pb biomonitoring

Under the procedures described above for digestion and analysis of bone samples and for the alkaline solubilization of the other tissues, all the samples were analyzed. These animals belonged to five different testing groups: 1—control animals ($n=3$), 2—animals intoxicated for eight days ($n=2$), 3—animals intoxicated for fifteen days ($n=3$), 4—animals intoxicated for thirty days ($n=2$) and 5—animals intoxicated for sixty days ($n=2$). Although the objective was to analyze at least three animals per group, due to difficulties regarding animal growth and also some ethical aspects, in some groups only two animals were used.

3. Results

3.1. Sample treatment

3.1.1. Acid digestion as sample preparation

Based on reported procedures for bone, pig kidney and bovine liver a unique sample treatment was evaluated for the different tissues of interest.

For bone samples, the literature suggests masses between 0.1 and 1 g of dried sample, which can be mineralized with nitric acid volumes varying from 5 to 10 mL [11–14]. These mixtures were heated in a microwave oven, using closed vessels and heating programs demanding 15 or 40 min [11–14].

For liver and kidney samples the procedures used are similar: masses from 0.02 and 0.50 g of dried sample were mixed with concentrated nitric acid [15–17] and hydrogen peroxide [18]. The volumes of nitric acid varied from 0.4 to 7 mL, while those for peroxide varied from 0.2 to 1 mL [15,16,18]. Finally, these solutions were submitted to microwave radiation with irradiation times between 13 and 30 min [15,16].

Considering this information, the available sample masses and the possibility of using diluted acid for sample preparation [19], a digestion procedure was optimized for 0.25 g of dried sample and 8.0 mL of 0.70 mol L^{-1} HNO_3. Regarding the microwave heating program, a slowly heating was used, due to the observation of relatively high pressures (near to 20 bar), depending on the tissue, in preliminary studies. Using the program shown in Table 1, the lowest pressure was observed for bone (10 bar) and the highest (20 bar) for the liver sample. In addition, the digests obtained presented similar aspects for the different tissues: the solutions were limpid, with no visible fat particles and then were considered adequate for direct use in the analyses.

3.1.2. Alkaline solubilization

Except for the bone and liver samples, only small amounts of tissues were available, depending on the age of the animals, especially in the controls and groups 2 and 3, mainly for heart and spleen. In these cases, an alternative and more convenient procedure was evaluated using alkaline solubilization.

Solubilization with TMAH was used for the different tissues and 1.00 mL of the reagent was enough for complete samples solubilization. The sample solutions presented a brown color and a large amount of foam was observed during homogenization but disappeared completely after a few minutes.

3.2. Instrumental parameters optimization

The following parameters were evaluated for the different tissues, aiming to obtain compromise conditions for the different samples: pyrolysis and atomization temperatures, use of chemical modifier and slit width.

In order to optimize the instrumental parameters the samples used were from a no intoxicated animal. The tissues were then fortified with Pb^{2+} in order to obtain sample solutions with 5.0 μg L^{-1} Pb^{2+}.

3.2.1. Conditions for the analysis of acid digests

In preliminary tests and different from the other tissues, heart and lung digests did not generate analytical signals, even when low pyrolysis temperatures (such as 650 °C) were used. Trying to understand this unexpected behavior, the sample digests were analyzed by ICP OES (employing an Optima 3000DV spectrometer, Perkin-Elmer) and ion chromatography (Metrohm Modular IC, Metrohm) in order to determine the main elemental constituents of the different samples and to verify possible interferences.

The data obtained in the elemental analysis indicated the presence of Al^{3+}, Ca^{2+}, Cu^{2+}, Cl^-, Fe^{2+}, Mg^{2+}, Mn^{2+}, Na^+, P (probably in the phosphate form) and Zn^{2+} in all the tissues but at different concentration levels. Among these elements, the highest concentrations were obtained for Cl^- (from 805 to 3170 mg kg^{-1}), Mg^{2+} (from 492 to 605 mg kg^{-1}), Na^+ (from 987 to 5171 mg kg^{-1}) and P (from 5908 to 56,153 mg kg^{-1}), suggesting that some correlation between their concentrations could explain the very low signals for the Pb^{2+} determinations in the heart and lung samples.

Evaluating these data, it can be supposed that the Mg^{2+} and P contents can act as a "natural" chemical modifier for these samples, affecting the atomization efficiency, especially by the amount of chlorine, which favors the formation of volatile chlorides. To verify this hypothesis the following ratio [amount of $(Mg^{2+}+P)$]/[amount Cl^-] was calculated for each sample and the values obtained were:

heart (202), lung (234), spleen (294), liver (562), kidney (584) and bone (7415). These results showed that this ratio was really smaller for the heart and lung samples than those for spleen, liver, kidney and bone. Thus, a chemical modifier was needed to avoid analyte loss from heart to lung samples.

Considering that the mixture $NH_4H_2PO_4/Mg(NO_3)_2$ is one of the most used chemical modifiers for lead, mainly when high pyrolysis temperatures are not necessary [20], this mixture was used in the analysis of all the tissues.

Using 50 μg of $NH_4H_2PO_4$ and 3 μg of $Mg(NO_3)_2$ as chemical modifier, pyrolysis and atomization curves were built for all the samples studied (Fig. 1).

Fig. 1I shows that all the tissues presented a similar profile under the pyrolysis step, except for the heart and bone samples. Then a compromise pyrolysis temperature of 850 °C was selected, based on these data.

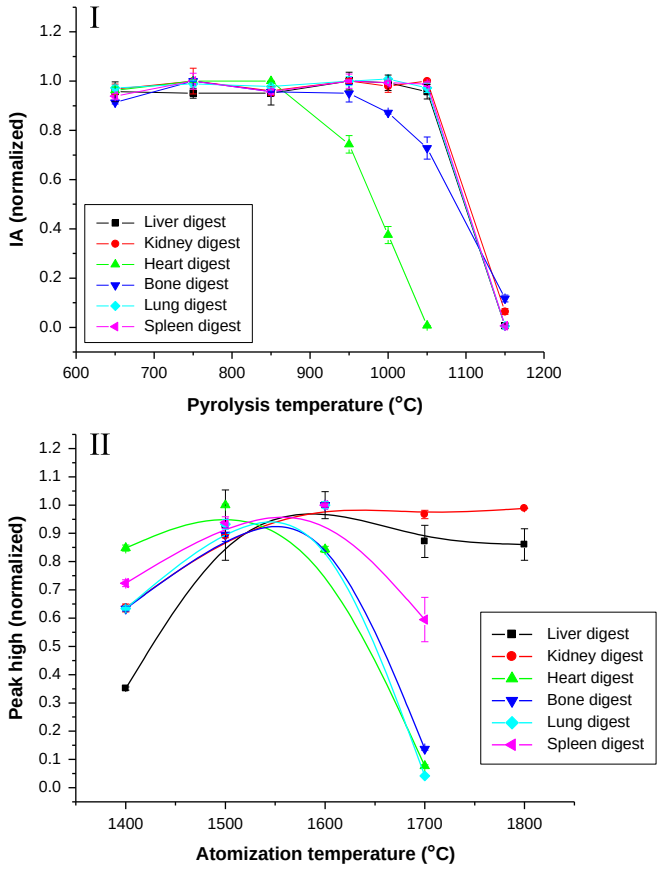

Fig. 1. Pyrolysis (I) and atomization (II) curves for lead determination in different animal rat tissues after acid digestion. (I) Temperature used for atomization: 1600 (°C) and (II) Temperature used for pyrolysis: 850 (°C).

By fixing the pyrolysis temperature at 850 °C the corresponding atomization curves were obtained (Fig. 1II). Aiming to evaluate the analytical signal and the peak profile for this study the signals were measured in peak height. In this case it was also necessary to choose a compromise condition, which was 1550 °C.

Under the selected conditions (use of chemical modifier, pyrolysis and atomization temperatures) the accuracy of the determinations was evaluated by analyzing samples of certified reference materials and using addition and recovery experiments. The results obtained are shown in Table 5.

Since the recovery values varied from 87 to 113%, the method studied presented good accuracies for the different tissues. On the other hand, it was necessary to use a smaller slit width for the analysis of bone samples. This fact may be explained by a high background interference, which is significantly minimized by reducing the slit width from 0.7 to 0.2 nm. Such an interference was also observed by Zong et al. [14], who solved this problem by using $Ca(NO_3)_2$ mixed with $NH_4H_2PO_4$ (as chemical modifier) instead of $Mg(NO_3)_2$ with $NH_4H_2PO_4$. Thus, this kind of "matrix matching" was tried and the results were also satisfactory. Regarding the other figures of merit (linearity and limits of detection and quantification) of the present study, they are presented in Table 6.

3.2.2. *Conditions used to analyze the samples after alkaline solubilization*

Considering that the introduction of solubilized samples may demand higher pyrolysis temperatures than those of digested samples, a chemical modifier must be used. The recommended chemical modifier for lead in such situations is the "universal chemical modifier" [20]. So, the pyrolysis and atomization temperatures were optimized by using $Pd/Mg(NO_3)_2$ as chemical modifier. The curves obtained are shown in Fig. 2.

Fig. 2I shows that the tissues of liver, heart and spleen, in TMAH media, are the most sensitive ones to the temperature increase. Thus a compromise temperature for the pyrolysis step was established as 950 °C, which corresponds to 100 °C above the temperature used for the digested samples.

Table 6
Linearity and limits of detection [a] (LOD) and quantification [b] (LOQ) obtained for the analysis of acid digests.

Slit width (nm)	Evaluated range (μg L^{-1})	Linear correlation coeficient	Instrumental LOD and LOQ (μg L^{-1})	Experimental LOD and LOQ (μg kg^{-1})
0.7[c]	1–100	$R^2=0.9991$	0.16 and 0.51	16 and 51
0.2[d]	1–100	$R^2=0.9968$	0.48 and 1.44	48 and 144

[a] The LOD was calculated as three times the standard deviation ($n=10$) [10] for a blank solution (HNO_3 0.2% v/v).
[b] The LOQ was calculated as nine times the standard deviation ($n=10$) [10] for the same blank solution (HNO_3 0.2% v/v) used for the LOD estimative.
[c] Slit width used to analyze heart, liver, kidney, lung and spleen samples.
[d] Slit width used to analyze bone samples.

Table 5
Recovery values obtained for Pb^{2+} in fortified samples (heart, spleen, lung) and reference samples (bovine liver, pig kidney and cheep bone) after acid digestion.

Tissue	Reported value	Found value	Added value[b]	Recovery (%)
Bovine liver NIST-1577B	0.129 ± 0.004 mg kg^{-1}	0.112 ± 0.007 mg kg^{-1}	—	87
Pig kidney BCR-186	306 ± 11 μg kg^{-1}	290 ± 4 μg kg^{-1}	—	95
Heart[a]	—	78.5 ± 7.1 μg kg^{-1}	150 μg kg^{-1}	100
Bone NYS RM05-04	30.0 ± 2.5 mg kg^{-1}	32.5 ± 0.1 mg kg^{-1}	—	108
Lung[a]	—	25.0 ± 6.9 μg kg^{-1}	161 μg kg^{-1}	113
Spleen[a]	—	142.6 ± 2.4 μg kg^{-1}	149 μg kg^{-1}	88

[a] Samples from an experimental rat, in which the "found value" correspond to the basal Pb^{2+} concentration.
[b] The added Pb^{2+} amounts (in μg kg^{-1}) varied for the different samples because the sample masses weighed were not the same and a fixed volume of a standard lead solution was used for all the spikes.

Using a temperature of 950 °C for the pyrolysis, the atomization temperature was evaluated for liver and heart tissues (in which the lead signal was shown to be less stable, during increasing temperature) and lung tissue (that presented the best thermal stability for lead).

According to Fig. 2II, small changes on the absorbance signals were observed, at about 13% among the evaluated atomization temperatures. On the other hand, considering that an atomization temperature of 1700 °C led to higher absorbance values and more precise signals, this value was selected for the atomization temperature.

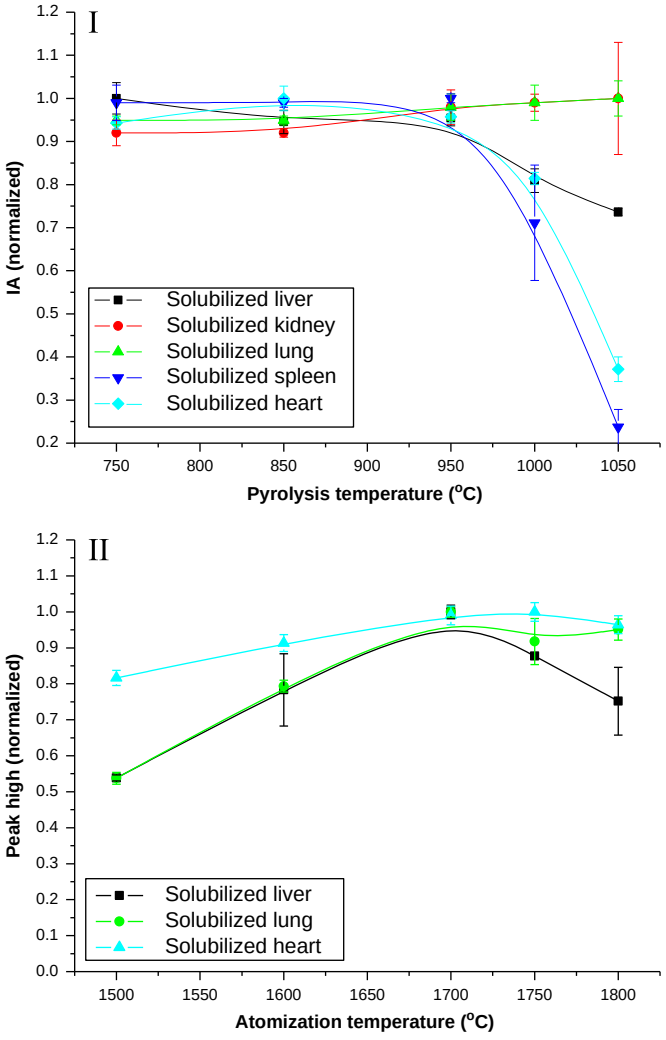

Fig. 2. Pyrolysis (I) and atomization (II) curves obtained for the sample tissues after alkaline solubilization with TMAH. (I) Temperature used for atomization: 1900 (°C) and (II) Temperature used for pyrolysis: 950 (°C).

Thus, employing the instrumental conditions optimized for the samples solubilized with TMAH (Tables 2 and 4), the method accuracy was evaluated by analyzing certified reference materials and also by addition and recovery experiments, as illustrated in Table 7. Good recovery values for lead can be observed, from 91 to 107%. These results show that the alkaline solubilization and the instrumental conditions used present good accuracies for the analysis of these kinds of tissues.

Under the optimized conditions the parameters linear range, limit of detection (LOD) and limit of quantification (LOQ) were evaluated and are presented in Table 8.

The method LOD and method LOQ values are related to sample dilution and are higher than those obtained for the acid digestion. However, this difference is small and the solubilization of rat organs with TMAH can be used without significant loss of detectability.

In relation to the sample and standard media, a comparison between analytical curves prepared in nitric acid (0.2% HNO_3) and TMAH showed that there are significant differences at the 95% confidence level. Thus the analytical curves must be prepared using the same alkalinity (with TMAH) as the samples.

3.3. Sample analysis for Pb biomonitoring

The optimized procedures for bone analysis (after acid digestion) and organs (employing alkaline solubilization) were applied for a group of 12 animals, evaluated as 72 different tissues, prepared and analyzed in duplicate, whenever possible (tissue dry weight higher than 50 mg).

Except for the control group, in which the analyte was not detected, all the other samples were diluted, as the corresponding sample solutions presented more than 100 μg L^{-1} Pb^{2+} (upper limit of the analytical curve). In this context it is very important to emphasize that different dilutions were used for the different tissue samples and even for animals of the same group. This fact showed that lead is significantly absorbed by some tissues although the concentration values showed a relatively high deviation between the replicates that corresponded to different animals.

The results are shown in Table 9, where the lead concentrations are also expressed in "accumulated percentage", that is, the total lead

Table 8
Figures of merit for the analysis of rat tissues after solubilization with TMAH.

Linear range (evaluated)	Correlation coefficient	Instrumental LOD* and LOQ*	Method LOD and LOQ
1–100 μg L^{-1}	R^2=0.9961	0.24 nd 0.71 μg L^{-1}	24–71 μg kg^{-1}

* The LOD was calculated as three times the standard deviation (n=10) [10] for a blank solution (TMAH 4% v/v) and the LOQ was calculated as nine times the standard deviation (n=10) [10] for the same blank solution.

Table 7
Lead concentrations obtained for the analysis of fortified (heart, lung and spleen) and reference samples (bovine liver and pig kidney) after alkaline solubilization.

Sample	Reported value	Found value	Added value (μg kg^{-1})[b]	Recovery (%)
Bovine liver NIST-1577B	0.129 ± 0.004 mg kg^{-1}	0.125 ± 0.007 mg kg^{-1}	—	97
Pig kidney BCR-186	306 ± 11 μg kg^{-1}	327 ± 4 μg kg^{-1}	—	107
Heart[a]	—	56.9 ± 4.3 μg kg^{-1}	280	100
Lung[a]	—	48.9 ± 0.6 μg kg^{-1}	241	93
Spleen[a]	—	58.5 ± 2.4 μg kg^{-1}	283	91

[a] Samples from an experimental rat, in which the "found value" correspond to the basal Pb^{2+} concentration.
[b] The added Pb^{2+} amounts (in μg kg^{-1}) varied for the different samples because the sample masses weighed was not the same and a fixed volume of a standard lead solution was used for all the spikes.

Table 9
Lead concentration determined by GF AAS in intoxicated animals.

Group	1: Eight days ($n=2$)		2: Fifteen days ($n=3$)		3: Thirdy days ($n=2$)		4: Sixty days ($n=2$)	
Tissue	**Pb content ($mg\,g^{-1}$)**	**Pb accumulated porcentage**	**Pb content ($mg\,g^{-1}$)**	**Pb accumulated porcentage**	**Pb content ($mg\,g^{-1}$)**	**Pb accumulated porcentage**	**Pb content ($mg\,g^{-1}$)**	**Pb accumulated porcentage**
Heart	0.0010 ± 0.0005	**0.12 ± 0.03**	0.004–0.30[a]	**0.11–0.58** [a]	0.17–0.37 [a]	**14 ± 3**	0.047–0.20[a]	**8–16** [a]
Lung	0.003–0.013 [a]	**0.26–1.15** [a]	0.014–0.556 [a]	**0.46–1.84** [a]	0.17–0.85 [a]	**14–36** [a]	0.08–0.37 [a]	**13–28** [a]
Liver	0.057 ± 0.020	**5 ± 1**	0.046 ± 0.004	**5 ± 1**	0.13 ± 0.02	**8 ± 2**	0.034 ± 0.002	**2–6** [a]
Kidney	0.086 ± 0.003	**8 ± 1**	0.11 ± 0.02	**15 ± 3**	0.16–0.42 [a]	**7–34** [a]	0.12 ± 0.01	**9–21** [a]
Bone	0.099 ± 0.003	**10 ± 1**	0.064 ± 0.002	**7 ± 1**	0.087 ± 0.016	**4–7** [a]	0.16 ± 0.01	**11–24** [a]
Spleen	0.76 ± 0.12	**76 ± 1**	0.59 ± 0.08	**71 ± 7**	0.32–0.79 [a]	**28 ± 5**	0.24–0.70 [a]	**31 ± 4**
Total accumulated lead	**1.01** $mg\,g^{-1}$		**0.82** $mg\,g^{-1}$		**1.84** $mg\,g^{-1}$		**1.13** $mg\,g^{-1}$	

[a] This concentration range corresponds to the lowest and highest lead concentrations determined for the replicates (animals) since the values varied significantly in the same group.

determined for the different tissues of each animal. This was a trial to normalize the data considering that the animals had absorbed different lead amounts, due to the different times of intoxication, but could have the analyte proportionally distributed among their tissues.

The data from Table 9 showed that the lead has accumulated mainly in the **liver, kidney, bone and spleen tissues** up to a period of fifteen intoxication days. Although the higher amounts were found for the spleen in this period, a decreasing trend was observed for this tissue and also for the bone. Concomitantly, the contents found for kidney showed an increasing trend (from 8 to 15%), comparing the groups from eight and fifteen days.

This behavior suggests that an adaptation process might occur, as the animals would be detoxifying themselves. Such hypothesis is reasonable since the kidneys are responsible for the blood impurities elimination [21] and also considering that the total lead accumulated in the different tissues during fifteen days ($0.82\,mg\,g^{-1}$) is 19% lower than the value accumulated during eight days ($1.01\,mg\,g^{-1}$). In addition, the increase in the lead contents for the lung and heart tissues along all the experiments indicated that the lead is being eliminated. Since this phenomenon is already known for bones [1], it can be considered that the lead accumulated in the spleen and also in the bones participates in a chemical equilibrium between "lead from tissues" and "lead from blood". At fifteen days this equilibrium is disturbed in the direction of increasing the lead amounts in the blood, favoring its elimination through urine.

This consideration could also explain the results obtained for the animals intoxicated for thirty days. In these animals the lead amounts found in the spleen and bone tissues continued to diminish while the lead amounts for the other tissues (kidney, liver, lung and heart) increased.

Thus, the results showed that the animals studied were capable of adapting themselves to a continuous lead exposure up to a period of sixty days of intoxication, when the experiment ended.

In addition, these data are in agreement with ones obtained in clinical studies for other animals submitted to the same intoxication treatment. In this case, the animals presented anemia and renal dysfunctions up to thirty days, after which the symptoms decreased and the animals started showing general health improvement between the thirty and sixty days.

4. Conclusions

Two analytical methods, based on the GF AAS technique, were evaluated and applied for lead determination in Wistar Hanover rats. One of the methods used an acid digestion, appropriate for bone analysis, and the other used an alkaline solubilization, which is feasible for the organ tissue samples and was more convenient due to the small amounts of each sample. For this latter procedure, TMAH was used for sample solubilization without heating or agitation. On the other hand, the analytical methodology showed that the conditions used to analyze the bone samples could also be employed for the other tissue samples.

Application of these methods allowed to analyzing the different tissues (bone, liver, kidney, spleen, lung and heart) in all the animals studied experimentally and the results gave new information on lead accumulation in rats. In fact, there was significant lead accumulation in the bones but other tissues, such as spleen, liver, kidney, lung and heart, were also involved in this process. Additionally, the animals seem to adapt themselves to the analyte exposure since the lead accumulation did not increase proportionally throughout the intoxication period. In this context the role of these organs was shown to be very important and complementary studies should be done aiming to understand the processes that could be stimulated to favor metal elimination in mammals.

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