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Original Study **Open Access**

A. Vicent, M.L. Cervera and A. Morales-Rubio*

Fast and accurate methodology for direct mercury determination in hair and nails

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Abstract: A solvent-free, easy, fast and waste-free methodology was developed for the determination of total mercury levels in hair and nails. Samples were taken from several volunteers and directly analysed, with levels of mercury in the range between 0.5 and 8 ng/mg. The influence of quantity of hair, as well as the addition of small amount of solvent and the necessity of previous treatment were studied. Also, a history of mercury exposure was provided by the distance of the hair from the scalp of each volunteer, the results of which were correlated with fish consumption. Furthermore, a short study of mercury in nails was carried out and correlated to the results from hair mercury levels. A small quantity of 5 mg of hair with an addition of 50 µL of water to the sample without previous treatment was adequate to get a representative result in less than 10 minutes. The accuracy of the proposed method was confirmed by analysing certified reference materials including Coal Fly Ash-NIST SRM 1633b, Fucus-IAEA 140 and three unpolished Rice Flour NIES-10. The observed results were found to be in good agreement with the certified values.

Capsule: A solvent-free and waste-free determination of total mercury in 5 mg hair and nails in less than 10 minutes.

Keywords: Total mercury, hair, nails, fish consumption

***Corresponding author: A. Morales-Rubio,** Departamento de Química Analítica (Universitat de València), Burjassot-46100 (Valencia), España, E-mail: angel.morales@uv.es
A. Vicent, M.L. Cervera, Departamento de Química Analítica (Universitat de València), Burjassot-46100 (Valencia), España

1 Introduction

Mercury and all compounds in which it appears combined are extremely toxic, especially to the nervous system, and its toxicity depends on the chemical form, quantity, method of exposure and vulnerability of the person exposed [1]. It is also known to bioaccumulate and biomagnify in the food chain [2]. Mercury is widely used in industry and people are exposed to it every day. There are coastal populations adjacent to industrial and mining areas activities; this proximity presents huge contamination problems to humans as waste containing mercury could spill into the environment and have lasting effects [3,4]. As a result, it is important to find a fast, effective and sustainable methodology to detect possible mercury contamination on biological or environmental samples.

Hair is a good sample to monitor Hg exposure because it can be sampled in a non-invasive and easy manner. Once it is incorporated into hair, mercury does not come back to the blood, and thus, it provides a good long-term marker of mercury exposure. Mercury concentration in hair and blood has been previously employed as valid biomarkers [5-7]. In fact, there is a direct relationship between hair and blood mercury levels, providing a confident method to measure Hg intake amount [8]. Mercury in hair remains stable and exposure over time can be observed in the hair strands. Provided that hair growth is approximately 1 cm per month, the Hg levels in the hair nearest to the scalp reflect a more recent exposure, while those farthest from the scalp are representative of past exposures [9]. The mercury fixed in the hair yields a signal that is approximately 250 times higher than that in blood and provides a history of exposure [1,10]. Several studies have employed Hg concentrations in nails as biomarkers for Hg exposure [11-13].

A number of studies have found a correlation between marine fish and seafood consumption and the level of mercury in hair [3,7,14,15].

Usually, once the hair is collected, it requires a long process in order to be analysed [16]. The hair should

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be washed and rinsed sequentially with non-ionic surfactant, high purity water and acetone before it is dried and digested [17]. The most frequently used technique for mercury determination is Cold Vapour – Atomic Absorption Spectrometry (CV-AAS) [9,18], due to its high sensitivity, high selectivity and relatively low instrumental cost. This method, however, involves the previous sample digestion and the introduction of high concentration of HCl and $NaBH_4$ solutions for the Hg vapour generation.

Therefore, a methodology that avoids the use of solvents and other reagents would prove far more attractive and result in an easier and more environmental friendly process. The direct volatilization of Hg through the sample ashed in an oxygen flow, followed by the pre-concentration of Hg in a gold trap could provide a direct and sensitive alternative for Hg determination of trace levels in complex samples. Employing a direct method also serves to avoid some problems found in multistep methods [19]. By using the direct mercury analyzer instrument, it is possible to determine the mercury concentration directly, even in solid samples like fish [20] and soil [21].

Moreover, the development of more environmentally friendly procedures is of great interest among analytical chemists [22,23] and the future lies in direct methods that use less resources (such as reagents and energy), and generate less waste. In this sense, the employed procedure in the present study is a good example of the so-called green analytical chemistry [24].

2 Experimental

2.1 Instrumentation

A Direct Mercury Analyser model DMA-80 (Milestone), for the direct analysis of mercury in samples, was employed. The instrument was equipped with an auto sampler with a capacity of forty sample boats; an interference filter of 254 nm with 9 nm width; and, a silicon detector, which measured the absorbance signal of Hg at 253.6 nm. The sample is weighed in a nickel capsule that is introduced into the instrument, and is combusted. The gases are carried by an oxygen flow through the catalyser to the gold coated sand where the mercury is selectively retained, while other products are flushed out of the system. After that, the amalgam is rapidly heated, releasing the mercury, and is analysed by a spectrophotometer reading the signals in two different cells for different concentrations. The procedure has been described in detail elsewhere [25].

The last calibration of the instrument was made three months before the study and, as a result, a new calibrate was prepared. Mercury solution was diluted to obtain an adequate range for the instrument and the expected results, which would be between 5 and 50 ng of mercury. The instrument provides a detection limit of 0.005 ng of mercury and a maximum Hg mass of 1000 ng. The efficiency of the instrument was checked by a standard addition and recovery study and by analysing certified reference materials.

2.2 Samples

Hair samples, from the scalp behind the ear, were given by a total of 18 volunteers after they had a bathing and were introduced into plastic tubes. The plastic material was previously washed in a 10 % nitric acid bath for 24 hours, rinsed with distilled water and allowed to dry. In addition, volunteers were briefly surveyed for later statistical analysis, as shown in Table 1. Nail samples were taken from only four of the volunteers. Each nail was numerated and analysed separately from the others.

2.3 Reference materials

Through the analysis of the certified reference materials, Coal Fly Ash 1633b (NIST-SRM) from the National Institute of Standards and Technology (Gaithersburg, MD, USA), Fucus sp. (seaweed) IAEA 140 from International Atomic Energy Agency (Vienna, Austria) and Rice Flour-NIES 10-Unpolished from National Institute of Environmental Studies (Ibaraki, Japan), the analytical validation of the method was carried out.

2.4 Procedure

Once the instrument was turned on, it was necessary to check the background signal after 15 minutes passing an oxygen gas current, at 200 mL min^{-1} through the system. An empty nickel capsule was introduced into the instrument and the process was completed without any sample. The reason of this first step was to clean the instrument from possible old contamination from previous analysis. When the mercury absorption peak was lower than 0.080 absorbance units, the instrument was ready to work and the analyses can be carried out. For direct determination of mercury in hair and nails, a small amount of sample was weighed inside the clean nickel capsule and introduced automatically into the direct mercury analyser (DMA-80).

A study of the influence of quantity of hair sample was done first. For this study, a larger sample of hair was provided from the same person. The hair was cut in very small portions and mixed to obtain a homogeneous

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Table 1. Information of volunteer hair donors

Hair sample	Age1	Gender2	Smoker	Fish3 (time/week)	Workers4	Length (cm)
1	24	M	No	3-4	Yes	< 5
2	58	M	No	> 7	Yes	< 5
3	45	M	No	1	Yes	< 5
4	25	W	No	1-3	Yes	10
5	27	W	No	< 1	Yes	8
6	28	W	No	< 1	Yes	6
7	23	W	No	5-6	No	12
8	24	M	No	1	No	< 5
9	22	W	No	1	No	12
10	49	M	No	3-4	Yes	5
11	13	W	No	1	No	10
12	29	W	Yes	2	No	12
13	23	W	No	2-3	No	12
14	33	W	Yes	2-3	No	11
15	25	W	No	1	No	12
16	57	W	No	2	No	12
17	24	M	No	0	No	< 5
18	22	W	Yes	1	No	8

1: Age in years. 2: man (M) or woman (W). 3: Times that volunteers eat fish a week.
4: If volunteers work at the Analytical Chemistry department.

sample, which was kept in a plastic tube for all the analysis. The aim of this study was to find the minimum necessary quantity of weighed hair to get a representative result. The amount of hair sample must be large enough so that the mercury can be detected and quantified. Amounts between 2.5 mg and 40 mg of hair were studied and the results compared. At the end of every experiment, each nickel capsule was cleaned with a brush and burned off with a blowtorch to eliminate any sort of interference for next analysis.

To improve the burning of the samples, these were moistened with a volume of ultrapure water added into the nickel cell. Quantities of ultrapure water between 0 and 60 µL were added to check the influence in the results accuracy. Several replicates were done for each addition of water.

Long hair samples were reserved for the study of the influence in the distance from the scalp. Each sample was cut with stainless scissors in 1 cm long pieces, and analysing them one by one. In case of having enough quantity of hair, a duplicated study of each centimetre was performed. In that case, the same weight of sample (5 mg) and volume of ultrapure water (5 µL) were added.

Different hair samples were pre-treated with acetone to test the requirement of a previous washing step. To a nickel capsule with 5 mg of hair sample, a volume of 0.2 mL of acetone was added and left in contact for 1 minute. After that, 0.1 mL of acetone was collected to be analysed. Both, hair and acetone were analysed to check the possibility of mercury extraction from the hair to the solvent.

2.5 Recommended procedure

No previous cleaning step was necessary and hair samples were introduced directly into the mercury analyser instrument. A quantity of 5 mg of hair, previously cut, was weighed into a nickel capsule and 50 µL of ultrapure water were added. The sample was analyzed by employing the time/temperature program shown in Table 2. The whole analysis lasted only 7 minutes. Regarding nails, the sample was analysed without addition of ultrapure water.

Table 2. Operating conditions for direct mercury determination

Step	Temperature (ºC)	Time (s)
Drying	250	60
Decomposition	250-650	100
Waiting	650	150
Amalgam	120	60
Record	900	12
	-	30

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3 Results and discussion

3.1 Optimization

The aim of this study was finding the best conditions for an accurate and representative analysis employing the minimum quantity of sample and solvents. Figure 1 shows the results obtained for a sample analysed several times by using different weight. As can be seen, the mercury concentrations in the different sample weights, from 2.5 to 40 mg, are near 4.60 ng mg^{-1}, and the dispersion improves with increasing weight of sample.

In order to improve the precision, another study regarding the influence of water addition on the sample analysed was performed in two different sessions. As it is shown in the Figure 2, the values of 20, 50 and 60 microliters of ultrapure water added to the hair sample provides lower dispersion of results, however, it was decided that the lowest quantity of water that completely covers the whole hair sample would be used. Because of that, 50 microliters of ultrapure water was chosen.

Regarding the necessity of a previous cleaning step of hair samples with acetone, the results shown in Table 3 demonstrate no significant difference between non-washed samples and those previously treated with acetone. Moreover, the acetone used in the cleaning treatment of hair sample was analyzed and mercury was not found. Therefore, it was concluded that treatment with acetone was not required.

3.2 Analytical parameters

The mercury analyzer has the capability to use two calibrations by means oftwo differently sized measuring cells. It was calibrated by using aqueous Hg standards. In order to measure low and high levels of Hg concentrations, two analytical calibration curves were constructed. The combined use of two cells in series allowed the measurement in the two ranges (typically 0–20 and 20–1000 ng of Hg). During the analysis, the same amount of Hg was measured twice, at the two different sensitivity levels, resulting in two instrumental dynamic ranges. The Hg content was then calculated automatically by the software of the instrument. The big cell was used to determine smaller quantities of mercury, calibrated from 5 ng to 20 ng of mercury (A = 0.0322(Hg) + 0.0677; R = 0.997); the small cell, which can analyse higher levels of mercury, calibrated finally from 20 ng to 50 ng (A = 0.000950(Hg) + 0.000426; R = 0.9999).

Recovery studies were carried out to check that the mercury present in the samples was being determined correctly without any loss or interference from the matrix. These experiments were carried out by employing of same amounts of three different hair samples to which a known mercury standard was added. Hair samples with different levels of mercury, low (sample hair 17), intermediate (sample hair 1) and high (sample hair 2) mercury content were chosen. The levels of added concentration, the mass of sample introduced in the system and the results are summarized in Table 4. As shown in the table, recovery values varied between 91 and 104%, indicating no significant matrix interferences during sample measurement. The repeatability of the methodology was evaluated by making six independent analyses of a hair sample 0.83 ng/mg Hg. The results found provided a relative standard deviation (RSD) of 7%. Additionally, the coefficient of variation (CV) for 3 independent

Table 3. Effect of acetone treatment on Hg levels

Hair weight (g)	Vacetone (µL)	(Hg) (ng mg^{-1})	Average
0.0050	0	0.7884	**0.83 ± 0.06**
0.0051	0	0.8753	
0.0049	200	0.7620	**0.83 ± 0.07**
0.0050	200	0.8189	
0.0050	200	0.9158	

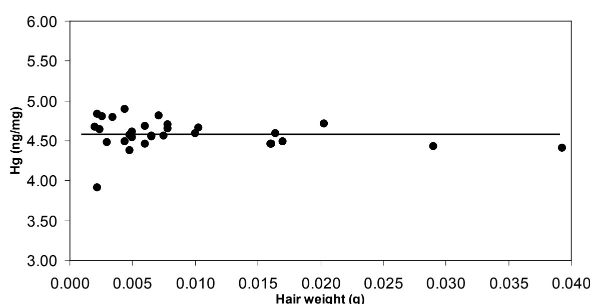

Figure 1. Hg levels detected in different weights of hair sample number 2.

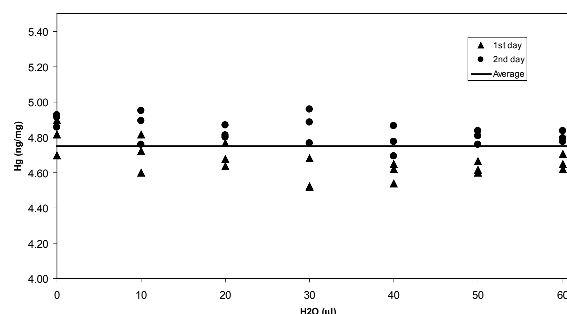

Figure 2. Hg levels according to different solvent addition (5 mg hair sample number 2).

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determinations of each hair samples varied between 1.2 and 25%, with the higher CV corresponding to the lower mercury concentrations.

The limits of detection and quantification (LOD and LOQ) were defined as three and ten times the standard deviation of ten measured blank concentrations respectively. For a hair mass of 5 mg, the LOD was determined to be 0.001 ng/mg and the LOQ was determined to be 0.004 ng mg^{-1} .

3.3 Validation of the method

Although a hair reference sample was not available in our laboratory, the proposed procedure was applied to the determination of mercury in other five certified reference materials. Coal Fly Ash-NIST SRM 1633b, Fucus-IAEA 140 and three samples of Rice Flour NIES- 10 (a, b, c) were employed. The comparison between the certified values of Hg and results found are in good agreement, as it can be seen in Table 5. In the case of rice samples, a reference value (not certified) was available; data rendered from analysis were of the same order as that reported.

3.4 Hair length study

The influence of the mercury level depending on the distance from the scalp was studied in the hair of several volunteers. It is known that the average hair growth is

Table 4. Recoveries of mercury obtained by using different hair samples (+ 50 mL of water)

Sample Hg content (ng mg^{-1})	Mass sample (mg)	Mercury added (ng)	Recovery* (%)
Hair sample 1	5	4	104 ± 1
0.973 ± 0.012	5	8	101 ± 4
	5	12	103 ± 7
	5	16	93 ± 1
Hair sample 2	5	5	99 ± 4
4.75 ± 0.11	5	10	100 ± 4
	5	15	95 ± 1
	5	20	97.7 ± 0.4
	5	25	97 ± 1
Hair sample 17	5	1	100 ± 13
0.105 ± 0.009	5	1.5	101 ± 4
	5	2	97 ± 8
	5	3	91 ± 9
	20	1	98 ± 1
	20	2	94 ± 5
	20	4	100 ± 3

*: Mean ± standard deviation (n = 3)

Table 5. Analysis of reference material

Reference sample	Certified concentration (ng mg^{-1})	Found concentration (ng mg^{-1})
Coal Fly Ash NIST 1633b	141 ± 19	138 ± 7
Fucus IAEA 140-TM	38 ± 6	35 ± 2
NIES Rice 10-a	4	6.0 ± 0.2
NIES Rice 10-b	3	3.98 ± 0.01
NIES Rice 10-c	5	6.45 ± 0.08

Note: NIES Nº10 a, b, c -Rice Flour-Unpolished- reference not certified value available.

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about 1 cm per month. Therefore determining mercury in different sections of the hair with respect to their distance to the root can get an estimated date of consumption of foods containing this element.

Figure 3 shows some results of the variation of the concentration of mercury found according to the distance to the root. Comparing those results with fish consumption of each volunteer, a high correlation was founded in all the samples.

3.5 Finger nails

Firstly, the equality of the mercury levels in every nail of each volunteer was proved by analysing each nail separately. As can be seen in Figure 4, there is no significant difference between mercury levels obtained in each nail.

Even employing only 4 volunteers for this study, it is possible to find a clear correlation between finger nail and hair mercury levels. Figure 5 compares the mercury

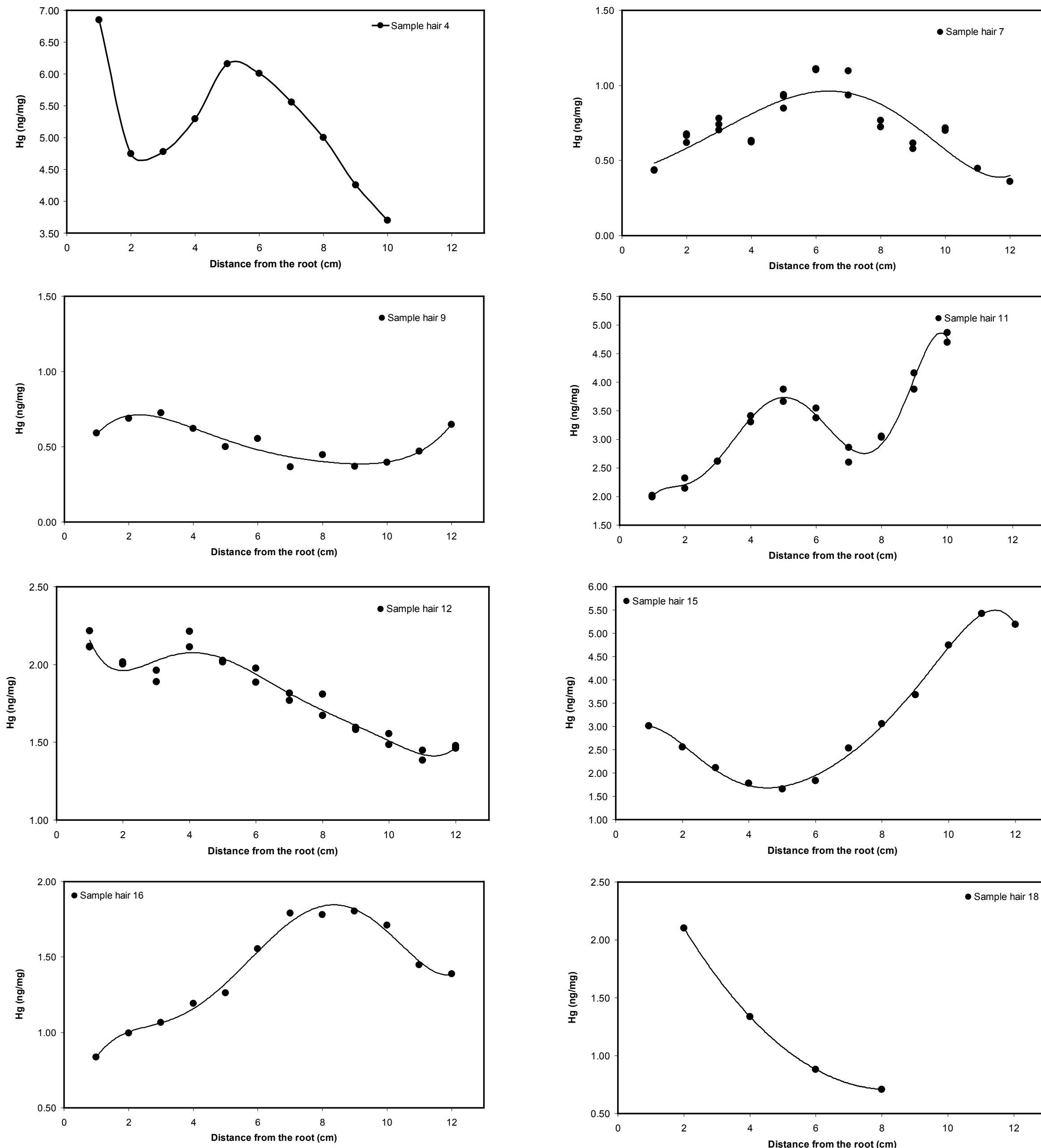

Figure 3: Variation of mercury concentration in hair compared to the distance to the root.

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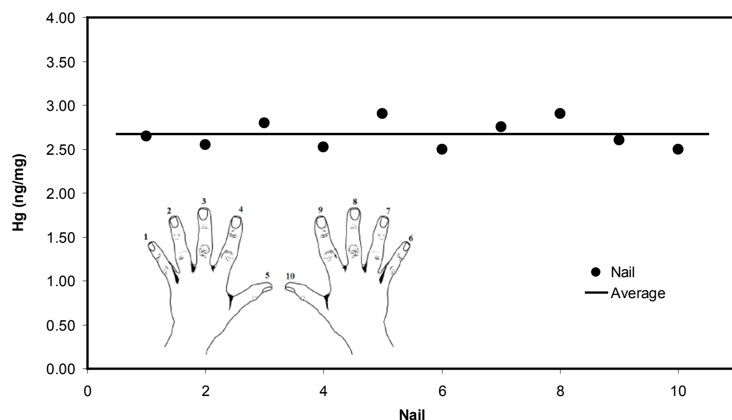

Figure 4: Hg levels in individual nail.

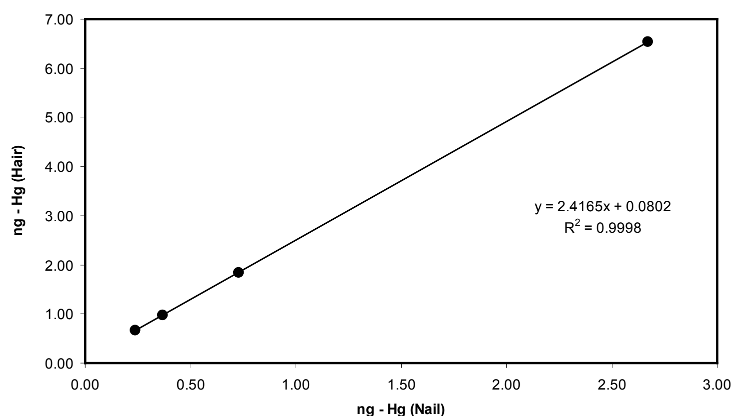

Figure 5: Correlation between Hg levels in hair and nails

concentration found in nails and hair and, as can be seen, a good correlation was achieved (0.9998), having 2.4 times more mercury in hair than in nails.

4 Conclusions

The major route of entry of mercury in humans is mainly due to fish consumption and, due to the Hg exposition, several neurological problems can appear. The proposed methodology could be a good, fast and accurate way to detect and measure any possible mercury contamination while employing a minimum quantity of hair and generating less waste. Even with a small quantity of nail, those mercury levels can be checked getting a representative value of the mercury levels in the population.

Employing the direct mercury analyzer as a screening technique is an excellent option. An amount of hair close to the scalp as small as5 mg is enough to get a representative mercury level. Less than 10 minutes is the required time to get the results, and adding 50 microliters of ultrapure water to get more accurate results.

In addition, the hair can provide evidence of past exposure and facilitates the estimation of when the exposure occurred. Furthermore, it is possible to employ nails as well as hair to get also a representative mercury level.

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