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Potentialities of total reflection X-ray fluorescence spectrometry in environmental contamination: Hair of owned dogs as sentinel of arsenic exposure ☆

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ABSTRACT

Arsenic concentrations in dog hair were measured in 50 pets living in Barrio Los Alamos, La Matanza district, Buenos Aires Province, Argentina. The aim of this work was to study the potential use of domestic canine hair as a biomarker of chronic exposure to arsenic by total reflection X-ray fluorescence spectrometry. Arsenic quantification in the samples was performed after a simple sample preparation procedure consisting in an in situ microwave digestion. Independently of genre, age and breed, hair of dogs from Los Alamos had significantly higher arsenic concentrations compared to a set of 10 dogs used as controls coming from an arsenic-free area. These levels found in hair (24 ± 2 μg As gDW^{-1}) indicate chronic exposure of dogs and suggest a similar situation in cohabitant humans. Results of this study encourage the potential use of pets as monitor targets of environmental metal contamination.

1. Introduction

Arsenic is a naturally occurring metalloid widely distributed among superficial and groundwater in Argentina due to the origin of the hydrogeological system [1,2]. At least 1.2 million people depend on untreated groundwater for drinking, with arsenic concentrations exceeding the recommended 10 μg As l^{-1} (WHO drinking water standard) [3,4]. This fact increases the risk of chronic exposure in populations consuming groundwater, leading to health consequences. The disease attributed to natural arsenic contamination is called 'chronic endemic regional hydroarsenism' (HACRE, 'hidroarsenicismo crónico regional endémico', in Spanish). Symptoms and health effects were studied and described by several physicians and toxicologists [5–7]. Thus, the need for development of new tools to monitor this pollutant is compelling.

Biomonitors are being used in many areas of environmental research and they are particularly useful to study exposure levels in a certain area. Biomonitoring gives a fullview of an internal or absorbed dose of a chemical in an organism and it can be the most reliable exposure assessment methodology as it considers all routes of uptake and all sources of contamination. Also, it reveals exposure history to a contaminant that might not be detected in water samples because of an intermittent source of contamination, making it an ideal instrument for risk assessment and risk management.

Animal testing has often been the basis for obtaining toxicity information in humans and for assessing the extent of contamination in the environment [8–10]. It provides information regarding human health effects as a result of exposure to the same environment (e.g. soil, sediments, water, air, and house dust) [11]. Since mammals share many physiological and biochemical characteristics with men, metals could have an impact on them in a similar way, acting as targets of contamination and allowing extrapolation [12].

Dog (*Canis lupus familiaris*) has long been an important research model and a promising tool as a target and bioindicator for metal contamination [13]. This is due to the fact that dogs are considered omnivores when associated with men [14] and share the same environment as humans, developing many of the same diseases [15]. Also, both dogs and humans share drinking water, but, unlike humans, who ingest waters from different sources, dogs consume only groundwater. In addition, canines are usually sedentary, consuming only the water meant to be evaluated.

Hair has been used as a biomarker in many environmental studies because trace levels of pollutants can be detected in a single strand [16]. Elevated levels of metals and metalloids in this appendage can indicate systemic poisoning [17–20]. Moreover, chronic exposure can be evidenced in hair rather than in fluids, because, unlike in blood and urine, arsenic accumulation is permanent [21]. Hair of owned dogs as a biomonitoring tool has also advantages over human hair because samples are easily obtained with minimum legal problems related to

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bioethics principles. In addition, samples do not require refrigeration for storage or transportation.

The most common analytical techniques available for arsenic quantification in such matrix are inductively coupled plasma with either optical emission (ICP-OES) [1] or mass spectrometry (ICP-MS) [16], flow injection hydride generation atomic absorption spectrometry (HG-AAS) [22] and voltammetry [23]. In these cases, in order to avoid matrix interferences and for the appropriate transport of the analyte to the atomization/ionization system, a laborious and time-consuming digestion is required. Moreover, these procedures produce an important amount of waste that needs to be treated. Total reflection X-ray fluorescence (TXRF) is a proven microanalytical technique for the analysis of metals at trace levels that avoids the inconveniences mentioned before. This technique is specially recommended for the analysis of highly toxic elements as it uses a small amount of sample [24,25]. Furthermore, sample manipulation and contamination are reduced by digesting it directly onto the reflector surface [26].

Canine hair samples as indicators of metal toxic pollution were mentioned by several authors. Dunlap et al. [14], studied sled dog's hair to link mercury exposure to human food systems in Alaska using cold vapor atomic fluorescence spectrometry. Arsenic concentration in tissues and body fluids of dogs was measured by Neiger and Osweiler [21]. In this study urine and hair were the best components for detecting low-level dietary inorganic arsenic exposure or poisoning. In Argentina, similar studies are scarce and they should be mandatory considering health risks.

The aim of this work was to study the potential use of domestic canine hair as a biomarker of chronic exposure to arsenic, through a simple microwave digestion method followed by TXRF determination using the Compton peak as internal standard [27].

To perform this study, dogs belonging to the Matanza district, Buenos Aires, Argentina were selected. This district shows high groundwater levels of arsenic.

2. Material and methods

2.1. Study site

The surveyed area was Los Alamos, an 80 km^2 neighborhood that belongs to the Matanza district, located 19 miles (31 km) away from the capital city in the Buenos Aires Province, Argentina (Fig. 1). Previous studies from this group showed that the selected area has elevated arsenic levels in groundwater, which is the main source of drinking water for the local population [28].

2.2. Sampling

Dogs sampled in this study belonged to Los Alamos neighborhood and were kept as companion animals. Homes selected for sampling were the same as those selected in the previous drinking water study [28]. Thus, a real correlation between exposure levels and hair concentration could be performed. Dogs were mainly fed on homemade foods or owner's leftovers. Stray dogs depending on the scavenging were not considered. Ten dogs coming from the capital city, drinking controlled tap water, were selected as controls. A total of 50 hair samples, coming from male and female dogs aged between 0.5 and 11 years, were collected. A lock of hair was taken from the lower neck area, near to the skin, cut with stainless steel ethanol-clean scissors. Samples were stored in clean plastic bags until sample digestion. All of the sampled dogs were clinically healthy and under normal food regime as reported by the owners. A survey was completed by the owners for each dog in their household during the sample collection procedure. The survey provided information on breed, age, gender, time of residence in the house, type of surface in the home yard (e.g., grass, cement), hours spent outdoors, and use of pesticides, medications or oral supplements that might affect hair arsenic levels.

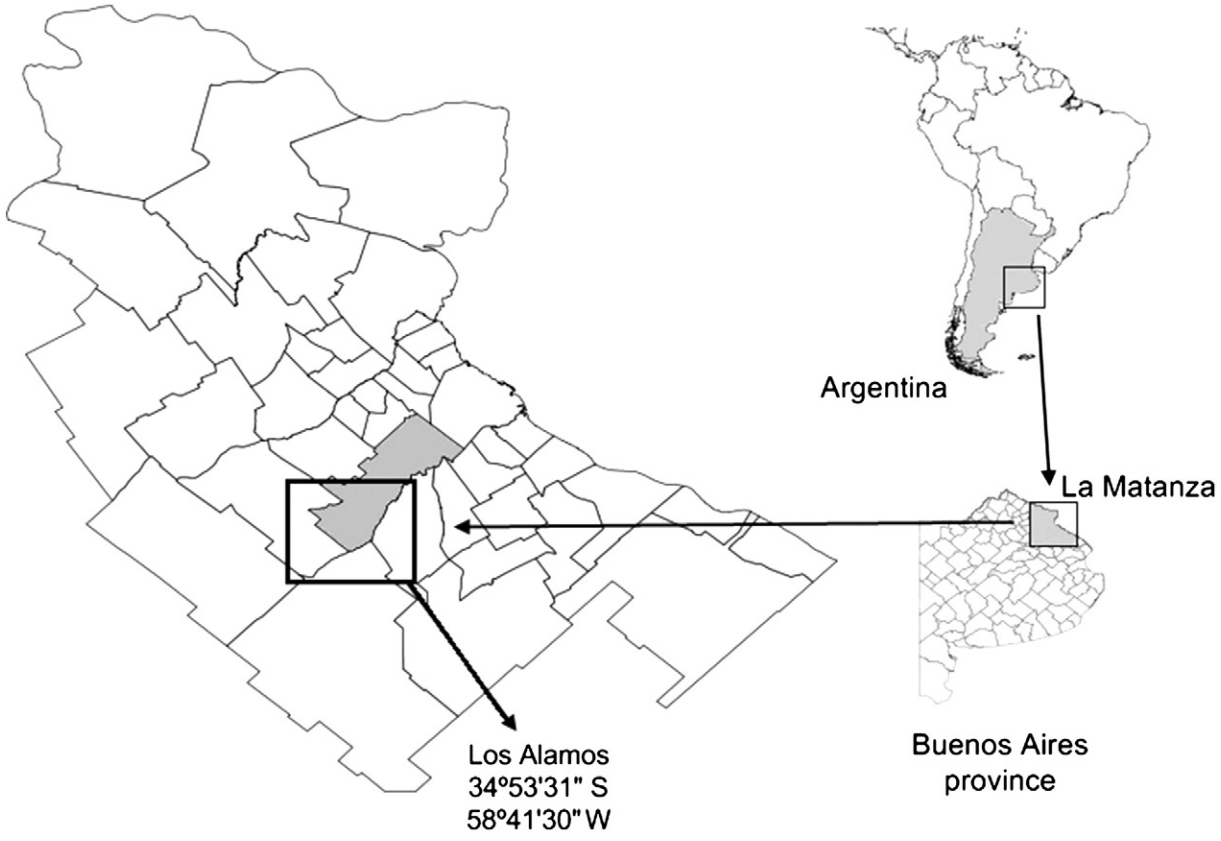

Fig. 1. Location of sampling site in La Matanza district, Buenos Aires Province, Argentina.

2.3. Reagents

All reagents used were of analytical reagent grade. A stock standard solution (Tritrisol® Merck, Darmstadt, Germany) of 1000 μg As ml^{-1} was used to prepare working solutions by serial dilutions with deionized distilled water (DDW). DDW was obtained by feeding with distilled water a Nanopure® Barnstead purification system (Dubuque, USA). A blank solution was prepared using only DDW. Nitric acid was prepared in-house by sub-boiling distillation of reagent grade material (70% Merck, Darmstadt, Germany) in a quartz still. A 30% v/v H_2O_2 solution was prepared with reagent grade material (Merck, Darmstadt, Germany).

2.4. Sample and standard preparation for TXRF and ICP analyses

Collected hair was soaked in 3% neutral detergent solution for about 10 min, rinsed with distilled water, defatted in acetone, and then rinsed again with distilled water and DDW. After removing moisture with filter paper, the hair was air-dried at room temperature, and stored in desiccators. A 1 mg hair subsample was placed on acid-cleaned quartz reflectors (30 mm in diameter) for in-situ digestion which was performed using a domestic microwave oven by adding 20 μl of 30% v/v H_2O_2 followed by 20 μl of 65% v/v HNO_3. The sequence of the digestion reagents used must be followed strictly in order to avoid the dispersion of the treated sample on the reflector surface. The reflector was then placed inside the microwave oven. The optimized digestion time was 8 min using the Defrost program and it was defined by the observation of total destruction of the matrix, observing a thin film on the reflector. The defrost cycle is 50% power, meaning that the full power is applied for half the time with no power applied the other half, being the average consumption 250 W. A control sample without hair was performed following the same procedure described above in order to consider blank contribution. For a more detailed explanation of the method, see Marcó P. et al. [26]. The sample was then irradiated in the total reflection spectrometer and the corresponding spectrum was collected. For quantification, the Compton peak was used as internal standard. A curve of relative sensitivity (*Ii*/*I*Compton) was drawn by fitting the obtained values with a second or third order polynomial function. For a complete description of the procedure, the interested reader is referred to Marcó P. [27]. Fig. 2 shows a typical spectrum of a digested sample.

Quantitative determinations were carried out by obtaining calibration curves for As (using five aqueous standards in 2% HNO_3) and analyzing the samples as described before. Calibration curves were checked every 10 samples and corrected if necessary. Confidence limits of interpolated value data were calculated by the software employed.

For ICP analyses, the digestion procedure was performed according Farias et al. [29].

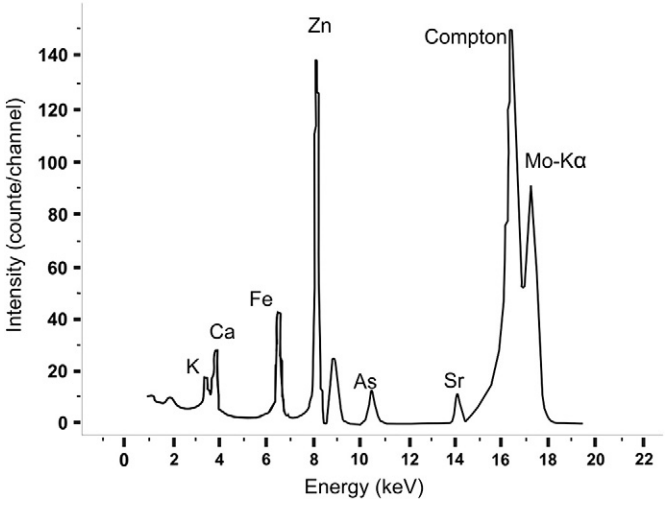

Fig. 2. TXRF spectrum of a digested dog hair from Los Alamos neighborhood using Compton peak as internal standard.

2.5. Instrumentation

A domestic microwave oven (BGH, R 120M20) was used for in situ digestion of samples. A METTLER analytical balance (±0.01 mg), model AE240, was employed for weighing the sub-sample. Digested samples were measured for arsenic by TXRF and the methodology was validated by ICP-OES of four randomly selected samples.

Tables 1 and 2 show the instrumentation and operating conditions used in the analyses. The TXRF spectral data evaluation was conducted with the AXIL fitting program and QXAS package supplied by the International Atomic Energy Agency.

2.6. Detection limits

For TXRF analyses, detection limits were calculated as three times the square root of standard deviation of the background as close as possible to the maximum signal. The detection limit (*DL*) was extrapolated according to Eq. (1), which assumes a 1000 s lifetime:

$$DL = \left[\frac{(N_b)^{3C}}{N_n}\right]\left(\frac{t}{1000}\right)^{\frac{1}{2}} \quad (1)$$

where N_b is the background intensity, N_n is the net intensity, C is the element concentration and t is the measuring time [30–32]. Peak and background were evaluated by means of the QXAS software.

The detection limit estimated for arsenic using TXRF was 3 μg l^{-1}.

2.7. Data analysis

Arsenic concentration in the hair of exposed dogs was compared against control dogs by Dunn's test as well as hair concentration sorted by gender. Correlation analyses between arsenic concentration in hair and groundwater were performed calculating the Pearson coefficient. Comparison between data obtained by ICP-OES and TXRF was performed by means of one way ANOVA. All statistical tests were done using STATISTICA 5.1 (Statsoft, Tulsa, OK, U.S.A.).

3. Results and discussion

Results obtained by both ICP-OES and TXRF are comparable for samples 1, 7, 13, and 22. No significant differences were observed for both techniques ($p = 0.485$) indicating that the proposed methodology is accurate (Table 3). Arsenic levels are found in hair of dogs from Los Alamos (μg gDW^{-1}). Data from ICP determination used for methodology validation are indicated for samples ACM1; 7; 13 and 22. No significant differences were found between methods ($p = 0.485$) by means of one way ANOVA.

No arsenic signal was observed in the blank solution, showing no contamination. Precision, expressed as confidence limit (%) was

Table 1
TXRF instrumentation and operating conditions.

TXRF Instrumentation	
Spectrometer	Canberra S-100
TXRF system	Atominstitut der Österreichschen Universitäten, Vienna
Excitation	Molybdenum anode K (17.4 keV). 40 kV and 20 mA
Detector	Si(Li) detector ($E_{Mn,\ 5.8\ keV} = 180$ eV)
live collection times	200 s.

Table 2
ICP-OES instrumentation and operating conditions.

ICP-OES instrumentation	
ICP generation system	HFP-2500 D (Plasma Therm, Kresson, USA)
Torch	Ames tyoe, PT-1 (Plasma Therm)
Nebulizer	Meinhard TR-30-A3
Spray chamber	Scott type, double barrel (glass)
Monochromator	VHR 1000, 1 m focal length (Jovin Yvon, Longjumeau, France)
Integration time	4 s
Analytical wavelength	193.69 nm 197 nm
Plasma operating conditions	
Forward rf power	1.25 kW
Frequency of rf generator	27.12 MHz
Coolant (outer) gas flow rate	151 min^{-1}
Auxiliary gas flow rate (intermediate)	0.61 min^{-1}
Sample (aerosol) gas flow rate	0.4–0.61 min^{-1}
Viewing height above load coil	12 mm
Continuous flow parameters	
Sample flow rate	2 ml min^{-1}
Sample acidity	0.3 M (HCl)
Tube size	1.5 mm (id)
Coil volume	750 μl

Table 3
Arsenic levels found in hair of dogs from Los Alamos (μg gDW^{-1}). Data from ICP determination used for methodology validation are indicated for samples ACM1; 7; 13 and 22. No significant differences were found between methods ($p = 0.485$) by means of one way ANOVA.

Sample	TXRF (μg gDW^{-1})	ICP (μg gDW^{-1})
ACM1	15 ± 2	16 ± 2
ACM17	11 ± 1	13 ± 2
ACM13	18 ± 3	20 ± 3
ACM22	15 ± 2	16 ± 2

better than 9.1% for As analyzed by ICP-OES and better than 14.3% by TR-XRF.

Data obtained from the survey concerning age, breed and gender of the sampled dogs as well as arsenic concentration in hair and groundwater are shown in Table 4. For control dogs, 70% of the cases showed arsenic concentrations below the detection limits. For the other cases, further investigations concerning diet, medication and water supply must be performed.

Table 4
Data collected from this survey. Average, median and range values for the age of the dogs, residence time, gender and breed was indicated. Average, standard error of the mean (SEM), minimum and maximum value are shown for arsenic concentration in hair (μg gDW^{-1}) and groundwater (μg l^{-1}).

	Los Alamos (n = 50)	Buenos Aires City (n = 10)
Age	Average 5 years Median 4 years Range 0.5–11 years	6 years 7 years 5–14 years
Residence time	0.5–10 years	3–12 years
Gender	Male (n = 36) 72% Female (n = 14) 28%	(n = 4) 40% (n = 6) 60%
Breed	Purebred (n = 0) 0% Mixed (n = 50) 100%	(n = 4) 40% (n = 6) 60%
Arsenic concentration in hair (μg g^{-1})	Average 24 SEM 2.4 Min 6 Max 70	Average 1 SEM 0.41 Min ND Max 4
Arsenic concentration in groundwater (μg l^{-1})	Average 61 SEM 1.3 Min 37 Max 76	–

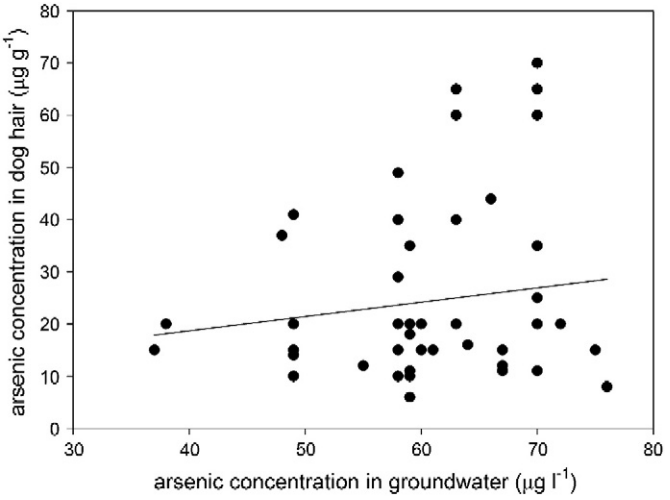

Fig. 3. Correlation between arsenic concentration in groundwater and in dog hair.

Arsenic concentration in hair of Los Alamos dogs was significantly higher than in control dogs ($p < 0.001$, See Table 4, 24 ± 2 μg gDW^{-1} for Los Alamos dogs and 1.0 ± 0.4 μg gDW^{-1} for control dogs).

In all samples, arsenic levels in groundwater exceeded WHO guidelines for drinking water quality [3] (Table 4). This fact is reflected in the high levels of arsenic found in hair, undoubtedly evidencing accumulation. Nevertheless, hair arsenic levels did not correlate with groundwater arsenic values ($p = 0.32$, $R^2 = 0.14$), as shown in Fig. 3. This could be explained because arsenic concentration in groundwater is variable and depends on several environmental factors, while arsenic in hair reveals chronic history and not punctual exposure.

Differences between male and female levels are significant for Los Alamos dogs ($p < 0.001$) as shown in Fig. 4. Arsenic levels in females were 65% higher than in males (average and SEM values are 46 ± 4 μg gDW^{-1} for females and 16 ± 1 μg gDW^{-1} for males) suggesting sex-dependent accumulation. A similar result was observed by Nowak in human hair [33]. This may be due to differences in keratin structure of hair fibers between males and females. More cystine in keratin of male hair was found by Clay et al. [34]. Also, sebaceous glands have less serum output in female hair than in male hair [35]. While differences in metal accumulation have been reported related to hair color, this fact was not considered in this study [36–38].

According to U.S. Department of Health and Human Services, arsenic levels over 1.00 μg gDW^{-1} indicate excessive exposure. According to

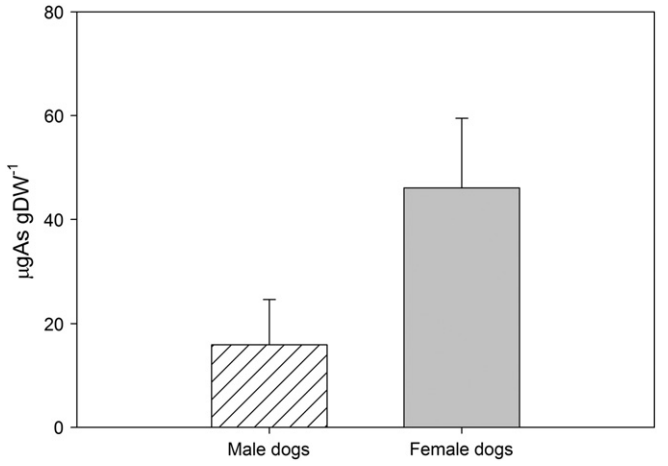

Fig. 4. Mean and SEM values for arsenic concentration in hair of male and female dogs from Los Alamos. Dunn's test showed significant differences ($p < 0.01$).

these guidelines, all sampled dogs in Los Alamos are chronically contaminated. Values obtained in this study exceed those considered by Neiger and Osweiler [21] as typical of background levels in unexposed dogs. These values are also higher than the registered for hair dogs from Bratislava (0.111 μg gDW^{-1}), Kosice (0.08 μg gDW^{-1}) and for the entire Slovak territory (0.091 μg gDW^{-1}) [39].

Concerning the technique, the advantages on this proposed methodology include an easy way of collecting and preserving the samples without being an invasive procedure to the neighbors. Also, it provides evidence of chronic exposition, even though contaminants are not detected in the water. It is also an efficient and clean method for digesting hair samples directly on the reflector and a fast, waste-free and sensitive technique for measuring arsenic levels.

4. Conclusions

The results of the present study demonstrate that there is arsenic accumulation in dog hair at Los Álamos neighborhood. This is an evidence of chronic contamination, probably due to the use of groundwater for drinking and cooking. These results serve as an alert for local population concerning arsenic exposure risks. Thus, the use of canine hair as sentinel of arsenic exposure is feasible. Additional studies are needed to demonstrate the same chronic effects in the local human population. Although the arsenic concentration in hair is useful for the detection of exposure, its use as an indicator of the degree of exposure on an individual basis must be carefully considered.

This study also showed that male and female dogs differ in their arsenic accumulation. Female dogs show more potential as sentinels due to their higher sensitivity to arsenic exposure. Hair color might also be an important factor to be considered when assessing the reasons for variation in hair accumulation.

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