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A Pilot Study Determining Hair Arsenic and Lead Levels in Residents of a Community Established on a Former Landfill in Puerto Rico

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Abstract Arsenic (As) and lead (Pb) were determined in hair of 12 adult women and 27 children living in a soil–metal contaminated community in Puerto Rico. Average concentrations in residential soils ranged from 24 to 109 µg/g for As and from 42 to 1,585 µg/g for Pb. For hair–As, about 75 % of the samples were below 0.08 µg/g. Hair–Pb presented levels of health concern, with 10 % of the samples above a reference value of 5 µg/g, indicating significant exposure to Pb. Multivariate analyses revealed that children's Pb hair levels were predicted by age and sex, while consumption of home-grown animals influenced the association with soil–Pb.

Keywords Arsenic · Lead · Hair · Puerto Rico

Arsenic (As) and lead (Pb) are widely distributed in the environment, and have been extensively recognized as environmental contaminants and human health hazards. Some of the toxic effects associated with inorganic As (toxic form of As) are: skin disorders, cardiovascular disease, and various types of cancer (e.g., skin, bladder, etc.) (ATSDR 2007a). Exposure to Pb has been linked with adverse health effects such as impaired intelligence and neurobehavioral development, hyperactivity, and anemia (ATSDR 2007b). Non-occupational human exposure to both metals can occur from soil, household dust, and water or food, among others. From the biomarkers often used to measure non-occupational exposure to As and Pb, hair is a good alternative for various reasons. Hair reflects chronic exposure (in terms of months), its collection is easy and noninvasive, and its storage and preservation involves minimal cost (ATSDR 2007a, b).

Between 1948 and 1979, more than 841,000 m^3 of commercial, industrial, and domestic waste were disposed and burned in approximately 0.29 km^2 of land used as a solid waste disposal site in the municipality of Vega Baja in northern Puerto Rico (USEPA 2010). Waste disposal operations were discontinued in 1979, while locals constructed homes in the area during the same time. This site includes a 0.22 km^2 community known as Brisas del Rosario (BR), with approximately 213 homes (USEPA 2010). Past soil sampling revealed concentrations of As and Pb as high as 190 and 14,000 µg/g, respectively (USEPA 2010). Since 1999, this metal-contaminated site has been included by the United States Environmental Protection Agency on the National Priorities List (NPL) of the Superfund Program (USEPA 2010). Given the high As and Pb soil levels in BR, there is a major health concern that residents from this community, especially children, are at risk. The objectives of this pilot study were: (1) to determine As and Pb exposure in adult women and children living in BR (a former landfill), as measured in hair, and (2) to associate metal levels in hair of children (the most susceptible group) with levels in soil and other behavioral and socio-demographic characteristics.

Materials and Methods

Subject recruitment (adult women and their children) occurred during April 2002 by visiting all houses included in a previous study (early 2001) conducted in BR (Sánchez-Nazario et al. 2003; Derieux-Cortés 2005). If

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still living in the house, adult women were informed about the nature of the study and those interested were asked to sign an informed consent form, authorizing the collection of hair samples (including from children), and the administration of a questionnaire to collect socio-demographic and behavioral characteristics.

Soil sampling and processing protocols have been previously described (Derieux-Cortés 2005). Briefly, composite top 5 cm soil samples were collected from each side of every participating house. After air-drying, individual composite soil samples were homogenized with a 230 μm sieve mesh, and digested in 10 mL of concentrated HNO_3 using a CEM microwave oven Model 1000 (EPA Method SW846-3051). After digestion, sample extracts were filtered through a Whatman 41 filter paper and diluted to 50 mL with distilled-deionized water (ddw). Individual composite soil samples from each side of the houses were analyzed for As and Pb, and the average concentration of each metal was determined per house.

Approximately 1 g of hair next to the occipital area of the head was cut with a stainless-steel scissor and collected in Whirl–Pack sterile polyethylene plastic bags. Prior to digestion, hair samples were washed to remove exogenous As and Pb (Senofonte et al. 1991). Briefly, about 0.5 g of hair was weighed and transferred to a beaker with approximately 60 mL of a 3:1 (v/v) ethyl ether and acetone solution, and left in a shaker for 10 min. The solution was discarded and hair samples dried at 85°C for 1 h. Then, samples were treated with 60 mL of a 5 % EDTA solution for 1 h, washed with ddw, and dried overnight at 84°C. Approximately 0.2 g of the washed and dried sample was microwave-acid-digested with 5 mL of HNO_3 during 40 min (CEM 1991), filtered, and diluted to 10 mL with ddw.

For quality control (QC), each digestion batch contained laboratory blanks, spiked blanks, and the standard reference materials SRM 2710 Montana Soil (NIST, Gaithersburg, MD, USA) and human hair powder GBW07601 from LGC Promochem, S.L. (Barcelona, España). Soil, hair, and QC samples were analyzed for As and Pb with a graphite furnace using a Perkin Elmer Atomic Absorption Spectrophotometer (AAS) Model A Analyst 800. Concentrations of As and Pb in soil and hair samples were expressed on a dry weight basis (dw).

Descriptive summary statistics as well as simple and multiple regression analyses were performed using STATA™ v. 10.0 (StataCorp, College Station, TX, USA). Levels of As and Pb in soil and hair were $\log_{10}$-transformed before performing regression analyses.

Results and Discussion

A total of 27 children and 12 women adults, corresponding to 11 houses, were recruited. Children's ages ranged from 2 to 17, while women's ranged from 23 to 55, with averages of 6 and 34 years, respectively. Participating children had been living in BR since they were born; and women for at least 3 years at the moment of sampling. Both groups reported spending an average of 2 to 3 h per week in the yard. Approximately 67 % of recruited children were female and near 82 % of children belonged to a family with a monthly income lower than \$800 (Table 1). A significant fraction of the children tended to chew toys (37 %), play with soil (48 %), and put their fingers in the mouth (54 %), as reported by the consenting adult. Eleven children (41 %), from 5 different houses, lived with at least one family member who smoked in their home. Other socio-demographic and behavioral characteristics are summarized in Table 1.

Average percent recoveries from digested spiked blanks were 96.9 % ± 2.3 % and 96.8 % ± 4.3% for As and Pb, respectively. Percent recoveries from SRM 2710 Montana soil averaged 98.0 % ± 3.4% for As and 95.4 % ± 4.5% for Pb; those from human hair powder GBW07601 averaged 91.7 % ± 11.5% for As and 86.9 % ± 8.5% for Pb. No significant contamination was found in laboratory blanks. Minimum reporting limits (MRL) of the AAS for As and Pb were 2 and 1 μg/L, respectively.

Average soil concentrations ranged from 24.2 to 108.8 μg/g for As and from 41.9 to 1,585.2 μg/g for Pb (Table 2). The United States has a national standard for Pb in bare residential surface soil of 400 μg/g (USEPA 2001). Approximately 50 % of the samples obtained Pb levels above this standard (Table 2). In the case of As, there is no national soil standard because of the wide variability on background As levels. Several states have established direct contact soil As standards based on background levels. For instance, standards for Massachusetts and New Jersey are 20 and 19 μg/g, respectively (Vosnakis and Perry 2009). In Puerto Rico there is no guidance value for As in soil. As observed in Table 2, all average As soil levels were above 19 μg/g. These results show the environmental problem that residents from BR confront because of the high residential soil Pb and As concentrations, and highlight the importance of conducting exposure assessment studies in this community.

Levels of As in hair samples of all adults ($n = 12$) and in 67 % ($n = 18$) of children's were <MRL. The maximum level measured was 0.17 μg/g, much lower than the reference level of 1 μg/g dw for As in hair (ATSDR 2007a). Low bioaccessibility and/or bioavailability of As from soil of BR may explain the low levels obtained in hair samples. Kientz et al. (2003) determined the bioaccessibility of As and other metals with soil samples from BR using synthetic gastric fluid, and obtained <10 % of bioaccessibility for As. Probably, this element is found in the form of chemical compounds that are not readily adsorbed by the body and are easily excreted (ATSDR 2007a).

In contrast, most hair samples showed Pb levels >MRL (85 % for children, 83 % for adults). Levels of Pb in hair ranged from 0.08 to 5.89 μg/g in children, and from 0.15 to 5.75 μg/g in adults (Table 2). A specific hair reference value for Pb does not exist (as it does for Pb in blood). In order to suggest reference values for this biomarker of exposure, different investigators around the world have measured Pb levels in hair of un-exposed (reference) populations. For instance, in Salvador, Brazil, an average value of 2.7 μg/g was determined in a population sample (Menezes-Filho et al. 2012). Park et al. (2007) suggested a value of <3 μg/g and a range of 4–5 μg/g for Korean preschoolers and for the general Korean population, respectively. Shah et al. (2011) found average hair Pb levels of 6.5 and 5.4 μg/g in Pakistani males and females, respectively. However, the background levels of Pb in these countries may not reflect what is found in Puerto Rico. A clinical laboratory in the USA (MML 2012) uses a value of <5 μg/g as the normal hair Pb content. In our study, approximately 10 % of the samples had hair Pb concentrations above 5 μg/g, indicating significant exposure to this metal. Kientz et al. (2003) found that the bioaccessibility of Pb from soils of BR (51 %–65 %) was significantly higher than for As, probably reflecting the higher hair–Pb concentrations.

Regression analyses were conducted only for Pb in children's hair for the following reasons: (1) children are the most susceptible population, (2) number of recruited women was low (n = 12), and (3) As levels in the majority of hair samples (children and adults) were <MRL. In the simple regression model, statistically significant ($p < 0.05$) positive relationships with Pb in hair were obtained for Pb in soil and with the toy chewing habit (Table 3). These findings are in agreement with other studies that have associated Pb in hair with environmental Pb levels (Sanna et al. 2003; Shah et al. 2011), and the habit of putting toys or fingers in their mouth (Kordas et al. 2010). In contrast, inverse statistically significant associations were observed for consumption of animals from the yard and having a family member smoking in the house (Table 3). Among the animals from the yard reported to be consumed by children (n = 9) (Table 1), chicken was the meat of choice for all of them. The inverse association of Pb–hair with chicken consumption may involve lowered intestinal Pb absorption

Table 1 Selected socio-demographic and behavioral characteristics of study population

Characteristic	n	Frequency	Percentage
Children			
Female sex	27	18	66.7
Household monthly income <$800	17	14	82.4
Daily floor cleaning	27	18	66.7
Window cleaning ≥ twice per month	25	18	72.0
Perceived low student achievement	25	9	36.0
Consumption of edible plants from yard[a]	27	14	51.9
Consumption of animals from yard[b]	26	9	34.6
Toy chewing	27	10	37.0
Playing with other children on the street	25	12	48.0
Eating soil from the yard	27	1	3.7
Playing with soil	27	13	48.2
Washing hands before eating	27	22	81.5
Putting fingers in mouth	26	14	53.9
Family member smoking in the house	27	11	40.7
Adults			
Female sex	12	12	100.0
High school education or less	12	10	83.3
Perceived low concentration	11	5	45.5
Currently smoker	12	2	16.7
Consumption of edible plants from yard	12	9	75.0
Consumption of animals from yard	12	3	25.0

[a] Includes fruits, vegetables, and seasoning plants

[b] Includes chicken, rabbits, and goats

Table 2 Distribution of As and Pb levels (μg/g dw) in hair of study participants and soil of their houses

			Min	25 %	50 %	75 %	90 %	Max
As								
	Hair[a]	Children (n = 27)	0.007	0.019	0.035	0.081	0.125	0.170
		Women (n = 12)	ND	0.0045	0.0095	0.017	0.023	0.050
		Overall sample (n = 39)	ND	0.011	0.025	0.064	0.122	0.170
	Soil	House average (n = 11)	24.2	43.85	58.81	71.80	78.97	108.80
Pb								
	Hair[b]	Children (n = 27)	0.081	1.169	1.807	3.195	5.536	5.892
		Women (n = 12)	0.148	0.2055	0.6595	2.139	2.798	5.745
		Overall sample (n = 39)	0.081	0.726	1.622	2.798	5.536	5.892
	Soil	House average (n = 11)	41.87	154.48	402.53	1,056.34	1,414.43	1,585.17

ND not detected

[a] 18 samples for children and 12 for women were <MRL for As (2 μg/L)

[b] 4 samples for children and 2 for women were <MRL for Pb (1 μg/L)

Table 3 Associations of children Pb concentrations in hair[a] with selected characteristics using simple linear regression analysis

Characteristic	n	Coefficient	Standard error	*p* value	R^2 (%)
Pb in soil[a]	27	0.2699289	0.1237239	0.0387	15.99
Age	27	−0.0342275	0.0204743	0.1071	10.05
Male sex	27	−0.3127146	0.1616449	0.0644	13.02
Family member smokes in the house	27	−0.3438227	0.1514019	0.0320	17.10
Playing with kids on the street	25	−0.2723195	0.16331	0.1090	10.79
Toy chewing	27	0.3256402	0.1561581	0.0474	14.82
Putting fingers in mouth	26	0.2714682	0.1584632	0.0996	10.90
Consumption of animals from yard	26	−0.4751978	0.1494492	0.0040	29.64
Anemia	26	0.3354669	0.2044256	0.1138	10.09
Constipation	26	0.3314046	0.2047015	0.1185	9.85
Asthma	26	0.2897934	0.1643247	0.0905	11.47
Urinary problems	24	0.3814456	0.2253205	0.1046	11.53

[a] Pb levels in hair and soil were $\log_{10}$-transformed

Table 4 Association of children's hair Pb levels[a] with selected characteristics using multiple regression analyses

Characteristic	Model 1 ($R^2 = 0.6812$; $p > F = 0.0026$)			Model 2 ($R^2 = 0.6362$; $p > F = 0.0018$)		
	Coefficient	Standard error	*p* value	Coefficient	Standard error	*p* value
Pb in soil	0.210	0.162	0.212	0.361	0.130	0.012
Age	−0.046	0.021	0.045	−0.048	0.021	0.030
Male sex	−0.384	0.131	0.009	−0.414	0.127	0.004
Toy chewing	0.248	0.133	0.079	0.256	0.131	0.065
Family member smokes in house	−0.042	0.167	0.802	−0.066	0.149	0.664
Putting fingers in mouth	−0.153	0.150	0.322	−0.147	0.151	0.344
Consumption of animals from yard	−0.285	0.185	0.142	–	–	–

[a] Pb levels in hair and soil were $\log_{10}$-transformed

in the presence of poultry-derived minerals like iron and/or calcium (Gundacker et al. 2010). The inverse association observed between Pb hair levels and environmental tobacco smoke ($\beta = -0.344$; Table 3), contrasts to what has been observed in other studies (Özden et al. 2007; Menezes-Filho et al. 2012). Some behavioral habits of the smoker (e.g., whether smoking occurred outside the house or the frequency of smoking at home), that could influence the association with Pb–hair, were not asked in the questionnaire. Neither was there information about the number of smokers living in the house.

Two multivariate models were fitted (Table 4) using selected predictive variables, based on statistical significance and on biological plausibility. Since the sample number was relatively low, it was necessary to reduce the number of variables included in our models, focusing it on non-illness variables that obtained a *p* value <0.1. Although the variable age was not statistically associated to Pb–hair levels in the simple regression analysis, it was included in both multivariate models because it is a potential numerical predictor of elevated Pb hair levels that is very commonly considered in these analyses (Sanna et al. 2003; Kordas et al. 2010; Shah et al. 2011; Menezes-Filho et al. 2012).

Multivariate Model 1 explained 68.12 % of the variation in children's hair Pb levels (Table 4). Soil–Pb was not associated with hair Pb levels in Model 1 ($p = 0.212$). However, when the variable "consumption of animals from yard" was removed (Model 2) the content of soil–Pb was found to be a significant contributor to Pb–hair levels ($p = 0.012$). Multivariate Model 2 explained 63.62 % of the variation in children's hair Pb levels, and it was also highly significant ($p > F = 0.0018$). This finding emphasizes the need for conducting further exposure assessment studies in this community, particularly on how children dietary habits (e.g., intake of home-grown animals) affect exposure to Pb.

The two consistent statistically significant predictors of elevated children Pb hair levels were younger age and being a girl, as shown in both multivariate models (Table 4). These results contrast with other studies, suggesting behavioral (habits) differences among children. For instance, Shah et al. (2011) found that older children and boys from Pakistan tend to have higher Pb hair levels. Sanna et al. (2003) observed higher Pb hair levels in boys than in girls from two Sardinian towns, Italy; but no association with age. Kordas et al. (2010) found that younger age in preschoolers from Uruguay was associated with Pb hair levels. Menezes-Filho et al. 2012 did not find a significant association between age nor gender with hair Pb levels of children from Brazil.

In conclusion, results showed that for two elements present in soils from BR, Pb represented a higher environmental risk than As for residents of this community. The multivariate analyses found that the variation in children's Pb hair levels was mainly explained by age and sex, and that consumption of home-grown animals influenced the contribution of soil–Pb content to hair Pb levels. Two main limitations of this pilot study were its small sample size and that it was not randomly selected, so that results cannot be generalized to the entire community. However, people in this community live in a site that is still included in the NPL of the Superfund Program because of its high concentrations of Pb and other metals in soils. Hence, due to the continuous exposure of residents from this community to high metal levels in soil, it is important to conduct exposure assessment studies, especially with children, to prevent potential adverse health effects.

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