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HIGH LEVELS OF HAIR MANGANESE IN CHILDREN LIVING IN THE VICINITY OF A FERRO-MANGANESE ALLOY PRODUCTION PLANT

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Abstract

Manganese (Mn) is an essential element, but an effective toxic at high concentrations. While there is an extensive literature on occupational exposure, few studies have examined adults and children living near important sources of airborne Mn. The objective of this study was to analyze hair Mn of children living in the vicinity of a ferro-manganese alloy production plant in the Great Salvador region, State of Bahia, Brazil and examine factors that influence this bioindicator of exposure. We examined 109 children in the age range of 1 to 10 years, living near the plant. Four separate housing areas were identified a priori on the bases of proximity to the emission sources and downwind location. A non-exposed group (n=43) of similar socioeconomic status was also evaluated. Mn hair (MnH) concentration was measured by graphite atomic absorption spectrometry (GFAAS). Possible confounding hematological parameters were also assessed. Mean MnH concentration was 15.20 μg/g (1.10–95.50 μg/g) for the exposed children and 1.37 μg/g (0.39–5.58 μg/g) for the non-exposed. For the former, MnH concentrations were 7.95±1.40 μg/g (farthest from the plant), 11.81±1.11 μg/g (mid-region), 34.43±8.66 μg/g (closest to the plant) and 34.22±9.15 μg/g (directly downwind). Multiple regression analysis on log transformed MnH concentrations for the exposed children derived a model that explained 36.8% of the variability. In order of importance, area of children's residence, gender (girls > boys) and time of mother's residence in the area at the birth of the child, were significantly associated with MnH. *Post hoc* analyses indicated 2 groupings for exposure areas, with those living closest to and downwind of the plant displaying higher MnH concentrations compared to the others. The contribution of the time the mother lived in the community prior to the child's birth to the children's current MnH suggests that *in utero* exposure may play a role. A study of neurobehavioral performance with respect to Mn exposure in these children is currently underway.

Keywords

Manganese; children; hair; environmental contamination; ferro alloy plant

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Introduction

Manganese (Mn) is an essential element, necessary for bone mineralization, energy and protein metabolism, cell metabolism regulation, and protection against oxidative stress (Keen et al., 2000). With normal dietary consumption, systemic homeostasis of Mn is maintained by both its rate of transport across enterocytes lining the intestinal wall and by its efficient removal within the liver (Papavasiliou et al., 1966). Exposure through inhalation is more effective than ingestion because Mn bypasses some of the homeostatic mechanisms that normally regulate its concentration in the body. In addition, animal studies have shown that inhaled Mn compounds can be taken up by the olfactory nerve and through axonal transport reach the olfactory bulb and other parts of brain (Brenneman et al. 2000; Dorman et al. 2006).

Neurotoxic effects resulting from excessive Mn exposure were first described by Couper in 1837 in Scottish laborers grinding Mn black oxide in a chemical industry (cited in Iregren, 1999). Neurological symptoms of manganism include decreased memory and concentration, fatigue, headache, vertigo, loss of equilibrium, insomnia, tinnitus, trembling of fingers, muscle cramp, rigidity, alteration of libido and sweating (Tanaka 1998). Studies of active workers with Mn exposure show diminished motor and cognitive functions, with changes in affect (for review see: Zoni et al., 2007). At even lower levels of exposure, studies of communities living in proximity to airborne Mn from mining and transformation activities have likewise reported neurobehavioral deficits (Mergler et al., 1999; Rodríguez-Agudelo et al., 2004), as well as changes in prolactin levels (Montes et al, 2008), associated with biomarkers of Mn exposure. Lucchini and associates (2007) showed a higher prevalence of Parkinsonian disturbances in a region of an Italian province with several ferro-manganese production plants, compared to other areas of the province.

Few studies have examined children exposed to airborne Mn. Blood Mn increases during pregnancy and Mn is actively transported across the placental barrier (Krachler et al. 1999). Smargiassi et al. (2002), who compared Mn concentration in cord blood from Montreal, where MMT was used as a gasoline additive, and Paris, with no MMT, reported that although there was no overall difference, there was a higher prevalence of high Mn in cord blood (defined as the 95^{th} percentile of the concentration in Paris:$\geq$ 6.8 μg/L), in Montreal. Takser et al. (2004) found higher blood Mn in pregnant women who reported that pesticides were used within a less than 1 km from their home.

Children’s hair Mn has been shown to increase with Mn intake from baby formula (Collipp et al., 1983) and drinking water (He 1994;Woolf et al. 2002;Agusa et al, 2006; Bouchard et al. 2006). This biomarker of Mn exposure has been associated with poor performance on neurobehavioral tests (He, 1994;Wright et al, 2006: Woolf et al, 2002) and with behavioral problems (Bouchard et al, 2006) for children exposed through drinking water or living near toxic waste sites. In Spain, Torrente et al (2003) did not find neurobehavioral deficits associated with hair Mn, but levels were lower than those reported in the other studies

Mn shares several characteristics with iron (Fe), both are transition metals, with valences of +2 and +3 in physiological conditions, relatively similar ionic radius. In addition, since Mn and Fe both strongly bind to transferrin and accumulate in the mitochondria, low Fe stores are associated to increased Mn uptake and retention in the blood (Roth et al., 2006). In a community study of adults, Baldwin et al 1999 reported an inverse relation between serum Fe and whole blood Mn. It has been shown that Fe deficient state (sideropenic anemia) is related to increased levels of Mn in blood (Mena et al. 1969).

The purpose of the present study was to determine hair Mn levels of children living in the vicinity of a ferro-manganese alloy production plant and identify factors that influence these concentrations.

Material and Methods

Study design and Population

The present study used a cross-sectional design in which we sought to compare children, aged 1 –10 years, living in different areas around a ferro-manganese alloy production plant and a non-exposed group of children of similar age. Because anemia and Fe status may affect Mn concentrations (Mena et al. 1969; Baldwin et al, 1999; Kim et al 2005), these hematological parameters were determined. The study protocol and consent procedure were approved by the National School of Public Health – Oswaldo Cruz Foundation Ethical Committee.

The plant, inaugurated in 1970 has an annual production of SiMn and FeMn alloys of 280,000 tonns. It is located in the metropolitan area of Salvador, capital of the State of Bahia, Brazil (See Figure 1) in the Cotegipe village (total population 620 inhabitants), a district of Simões Filho with 109,269 inhabitants (IBGE 2007). The area is separated from the urban area by the BR-324 highway. The Centro Atlântica Railway, which brings in raw material to the plant and transports the ferro-manganese lingots to the Aratu harbor, passes through the village. The plant's geographical coordinates are 12°47′18″S and 38°24′41″W.

Air Mn in $PM_{2.5}$ was measured over 24 hour period during seven days in August 2007. The air sampler was installed on the roof of one house (geographical coordinates 12°47′23″S and 38°25′24″W) located 1.3 km from the plant and about 70 m from the school and the daycare center. Sampling was performed using a Cyclone URG (URG 2000) coupled to a vacuum pump adjusted to 10 L/min flow. The 47 mm diameter quartz membranes (SKC®) were extracted according to the EPA procedure (Compendium Method IO-2.1. EPA). Field and reagent blanks along with spiked samples were analyzed. The average and median Mn concentrations during this rainy period were 0.151 $\mu g/m^3$ and 0.114 $\mu g/m^3$, respectively (range 0.011– 0.439 $\mu g/m^3$).

In July 2006 we carried out a census in which we listed and geo-referenced 154 houses regularly inhabited by 165 children from 1 – 10 years of age. Information obtained from a community leader indicated that the village was spread along the road and divided into three sectors. We decided to include the residents of Virgínio Dame Street, an unpaved 2-Km road on the north-west side of the industry, who complained of heavier dust fall-out on their residences. This is an estuarine area mostly plains with small hills; yearly wind prevalence is from southeast to north-west (VEEP, 2005). Spatial stratification, based on the distance and geographical position on relation to the plant's chimneys, was used to identify *a priori* distinct exposure areas (Figure 1).

- Area A – Houses along a road located on the edge of the south-west side of the plant fence, at an average distance of 0.6 Km. A total of 16 children (9.7%) live in this area
- Area B – The village center located at an average distance of 1.5 Km west of the plant. It is more densely inhabited. Daycare and elementary schools are located here. We identified 108 (65.5%) children living in this area
- Area C – This area is located approximately 1.6 km from the plant towards the Southwest on a plateau, approximately 80 meters high. We identified 19 (11.5%) children in this area.

- Area D – This is an isolated community living downwind along a west-bound road ranging from 0.9 to 1.7 Km from the plant. Twenty-two children (13.3%) children were identified in this area.

The non-exposed children lived in the community of Capiarara, in the municipality of Lauro de Freitas town, located 7.5 Km southeast from the plant in an upwind direction. A census of the whole community, performed in March 2008, identified a total of 379 inhabitants (103 children in desired age range).

In the exposed community, the local economy is based on the cultivation of cassava and rudimental processing and commercialization of manioc flour. In the reference community, the main income is from informal jobs in the nearby wholesale vegetable and fruit market.

Recruitment

In April 2007, a meeting was held with the community living near the plant. The objectives and procedures of the study were explained. Written informed consent was obtained from the parents of the 145 (87.9%) children who were still living in the region. In June, 2007, a total of 109 (75.1%) children within the selected age range provided hair and blood samples.

Children in the reference community were recruited following a census. We visited each household and invited the parents or the care-giver (when not a parent) to participate in the research. Parents of 76 children agreed to participate in the study, providing written consent. On the day of biological sampling 49 children (64.5%) were available to provide blood and hair samples. Due to the fact that 6 boys had their hair shaved to the scalp, we couldn't collect samples, final sample size consisted of 43 children.

Data Collection

Hair sampling and analysis—A tuft of hair of approximate 0.5 cm diameter was cut off with a surgical stainless steel scissor as close as possible to the scalp in the occipital region, after tying with a Teflon string at the proximal end. For boys with short hair (less than 2 cm in length), an equivalent amount was trimmed directly into the sterile sampling plastic bag. After identification with the proper child code, the sampling bags were stored at room temperature until analysis. In the laboratory, hair samples were washed according to the procedure described by Wright *et al.* (2006). Briefly, the first centimeter or the amount available was washed for 15 min in 10 ml of 1% Triton X-100 solution in a 50-ml beaker in ultrasonic bath. Rinsing was performed several times with Type I pure water (Milli-Q, Millipore®). Hair samples were dried wrapped in Whatman N#1 filter paper in a drying oven at 70°C overnight. Approximately 10 mg of hair was weighed in 50-ml beaker and digested with 2 ml of spectroscopic grade concentrated HNO_3 acid for two hours on an 80°C hotplate. The digest was then diluted to 10 ml with Type I pure water in a polypropylene centrifuge tube (Corning ®).

Acid digested samples and reference material were analyzed using electrothermal atomic spectroscopy with Zeeman background correction (GTA-120, Varian Inc.). All glassware and plasticware were thoroughly decontaminated by soaking for 24 hours in 3% neutral detergent (Extran®, Merck), followed by soaking overnight in 10% HNO_3 and finally rinsed with Type I pure water. Reagent blanks were analyzed along with samples in every batch. The detection limit was 0.1 μg/L. Routine checks of accuracy and precision were accomplished using human hair reference material from the International Atomic Energy Agency (IAEA-085). The intra-batch and batch-to-batch precisions were 4.4% and 5.1%, respectively. Accuracy in the concentration range of 8.3 to 9.3 μg/L was 103.2%. All samples and SRM were determined in duplicates and a difference lower than 10% was considered acceptable.

Anemia—Blood samples were drawn by venipuncture into two different vacutainer tubes, one with EDTA for determination of hemoglobin (Hb) and cell counting by automated equipment (Hematology Analyzer Pentra 80, ABX) and the other with no additive for serum Fe determination by colorimetric method using a commercial kit (Roche Hitachi 747, Roche®). We applied the WHO criteria for anemia: For children under 6 years of age, we used Hb levels ≤ 11.5 g/dL and for the older children ≤ 12.0 g/dL (WHO, 1994).

Socio-demographic information—Parents or care-givers responded to a socio-demographic questionnaire, administered by trained interviewers. One questionnaire included information on socio-demographic characteristics of the family (housing structure, educational level, time living in the community etc), general habits (consumption of water and vegetables grown locally). A second questionnaire focused specifically on the child and included general information on development, education, health status and recreational activities.

Data analysis

Each child was coded with respect to area of residence and house number. Descriptive statistics were used to determine the distribution of socio-demographic information, hair Mn and hematological parameters.

Frequency distributions were compared using Fisher's exact test. Normally distributed continuous variables were compared using the Student t test, while for variables that were not normally distributed, Mann-Whitney (MW) or Kruskal-Wallis were used depending on the number of categories.

Since the distribution of hair Mn was skewed, data were log10 transformed for further analyses. Backward stepwise regression models were used to identify variables that were potentially associated with hair Mn (0.100 to enter; >0.05 to exclude). These variables were then included in a linear regression model. A Tukey *post hoc* test was used to determine inter-area differences. A significance level (p=0.05) was used. All statistical analyses were performed using SPSS version 13 software.

Results

Population characteristics

Table 1 presents a summary of the study populations' main characteristics. Both communities are ethnically comprised of a majority of Afro-Brazilians. The large majority has dark hair. In the exposed community, for 70.3% hair color is brown to dark brown; 28.7% are black haired and one child is blond. In the reference community, all have dark brown to black hair. They are low income families, with an average monthly stipend of U$ 150. All families receive a federal government stipend per child enrolled at school (*Bolsa família* program). The majority of those who responded to the questionnaire was the biological mother (85% in the exposed *versus* 76% in the referents); 7% and 14% were fathers and 8% and 10% care-givers (grandmothers or godmothers), respectively. Parents and caregivers reported a low number of years of formal schooling (mean < 3.0 years).

For the children, gender proportions were similar in the exposed (48.6% boys) and reference (42.9% boys) communities. Children from both communities did not differ in age, hemoglobin and serum iron levels. In the exposed group, 21 of the 106 children (19.9%) were classified as anemic; the prevalence of anemia in the reference community was 18.8% (9/48). This difference is not statistically significant (p=0.533).

Children's Mn hair levels

Box plots of MnH data according to area of residence, clustered by gender are in Figure 2. For the reference group, geometric mean and median MnH concentrations were 1.13 μg/g and 1.19 μg/g, respectively, ranging between 0.39 and 5.58 μg/g. A total of 7% (3 children) surpassed 3.0 μg/g. Geometric mean and median MnH for the children living in the vicinity of the plant were 9.96 μg/g and 9.70 μg/g, ranging from 1.10 to 95.50 μg/g; the large majority (91.7%) of MnH levels was above 3.0 μg/g. Girls MnH levels were significantly higher than boys. For the reference group, median levels were: 1.59 μg/g vs 0.95 μg/g. respectively, (MW $p=0.023$), while in the exposed group girls presented a median concentration of 13.78 μg/g and boys: 6.56 μg/g, (MW $p<0.001$). Among those who lived near the plant, 88.1% were born in the community and no difference was observed in MnH levels between the children who were born there and those who were not (boys born in the community 10.70 μg/g; others 13.47 μg/g; girls born in the community 19.21 μg/g, others 18.88 μg/g).

No correlation was observed between MnH with age or with hemoglobin or serum iron levels). For those with anemia median MnH for the reference group was 1.35 μg/g vs 1.15 μg/g for those who did not present anemia. For the exposed group, these median concentrations were 9.20 μg/g and 9.70 μg/g, respectively.

Table 2 shows MnH levels with respect to the four residence areas. The highest levels were observed in Areas A and D, which correspond to residences closest to the plant and those directly downwind. Gender differences (girls>boys) were present at every location. No differences were observed in children's age between areas of residence (ANOVA, $p>0.05$).

Table 3a presents the results of the multiple regression model for log MnH with only the exposed children. Gender and area of residence enter significantly into the model, explaining 26.9% of the variance. *Post hoc* tests showed that residence area could be grouped into two with those residing next to the plant or downwind with the higher MnH concentrations (Areas A and D) and those in the centre of the village and the outskirts displayed lower levels (Areas B and C). Both are significantly different from the control group.

We explored the influence of time of mothers' residence in the area at childbirth as a possible surrogate of *in utero* exposure. For the exposed group, maternal exposure duration before child's birth was obtained by subtracting the age of the child in years from the time the mother reported living in the area near the ferro-manganese alloy production plant. The average time of mothers' residency in the area was 8.0 years and ranged from zero (for those children who were not born there) to 29 years. When mothers' time of residency in the area was included in the above model (Table 3b), the t-values for gender and area of residence were basically unchanged and the model explained 36.8% of the variance of log MnH. The model respected the linear regression assumptions and the standardized residues displayed normal distribution (mean=zero and SD=0.986). Figure 3 shows a scatter graph of the residual plot of log transformed MnH level with respect to years of maternal exposure time previous to child's birth, the partial correlation coefficient shows that this variable alone explained 12.1% MnH variation.

Discussion

The levels of MnH found in the exposed children in this study are on average ten times higher than those in the non-exposed children. MnH reference value for Brazilian adults (0.15–1.2 μg/g) has been previously determined using inhabitants of Rio de Janeiro in a sample of 1,091 men and women (Miekeley et al, 1998).

In Cotegipe village, Mn concentrations in raw and drinking water were 74.4 ± 8.63 μg/L and 27.7 ± 15.02 μg/L, respectively (data not published). These values are relatively low compared to the WHO guidelines of 400 μg/L (WHO, 2006). In the region surrounding the manganese alloy production plant, air Mn appears to be the major determinant of children's hair Mn. Air Mn concentrations in ultrafine particles ranged from 0.011 to 0.439 μg/m^3 at the sampling site, located in the core of the community, 1.3 Km from the plant. Lucchini and coworkers (2007) measured Mn in the respirable fraction, using a similar technique, in six locations within 2 Km from a manganese alloy plant with similar processes to the one here. They reported a geometric mean of 0.69 μg Mn/m^3 (range 0.2–1.8 μg Mn/m^3). The authors indicated that in the metropolitan area of Brescia, about 50 km downwind from the alloy plant, Mn concentrations were 0.08 μg Mn/m^3 and ranged 0.050–0.30 μg Mn/m^3. In the present study, we were unable to perform air environmental monitoring in all four areas due to equipment availability and limited budget.

The findings of the present study suggest that the main source of airborne Mn exposure is the fumes from the alloy plant chimneys. A clear pattern was observed when MnH levels were analyzed spatially, with the highest concentrations in those children who lived closest to the plant or in the downwind direction.

MnH levels observed in this study are the highest concentrations reported in children environmentally exposed to manganese (He 1994;Woolf et al. 2002;Wright et al, 2006; Bouchard et al. 2006). However, measurement techniques may differ from one study to the next. A panel convened by ATSDR (2001) to provide guidance for agency health assessors on the use and interpretation of hair analysis data emphasizes that although the technology exists for assessing substances in hair, variations in sample collection, preparation, and analytical methods can drive what will be measured in the final analysis. It has also been suggested that Mn may be more readily found in darker colored hair (Lydén et al., 1984;Sturaro et al., 1994). The exact mechanism of Mn uptake in hair follicle has not been fully elucidated (Robbins CR, 1994), but it is well known that Mn has a high affinity for all types of melanins encountered in hair, skin, iris and in the CNS (Lyden et al. 1984). A report of MnH concentration among adults in Cotegipe village indicated average concentrations between 66.38 and 177.43 μg/g, depending on the area of residence (VEEP, 2005), but hair sample collection protocol, washing procedure and analytical methods were not indicated. In the present study, since Mn exposure is mainly through airborne route, it could be argued that the high Mn level observed in this study could be due to external deposition. It should be noted that extra care was taken in the hair sample treatment. The procedure described by Wright et al. (2006) was adopted to wash hair samples, because it applies a mild detergent in ultra-sound bath for 15 minutes. This procedure yields a thorough hair wash without destroying hair structure.

In this study, girls had significantly higher Mn hair levels than boys. Similar results were reported by Bouchard et al. (2007) for boys and girls exposed to high levels of Mn in well water in Québec, Canada. Wright et al. (2006) evaluated 31 children living near a waste site in Oklahoma, USA and found no significant difference between hair Mn levels in boys and girls, but hair Mn concentrations were considerably lower (mean 0.47 μg/g) than those observed in the present study or in the study by Bouchard et al (2007). In a community-based study of adults, Baldwin and associates (1999) observed that women presented higher Mn blood levels than men. The authors suggest that men and women differ in Mn metabolism, which may be related to Fe status.

Due to its electrochemical similarity to Fe, Mn competes for the same transport mechanisms for intestinal Fe absorption, where both bind to the divalent metal transporter-1 (DMT1), Thus Mn may be absorbed more efficiently when there is a depletion of Fe stores (Garrick and Dolan,

2002; Roth, 2006). Although, anemia was present in approximately 19% of the children, there was no difference in MnH with anemia status, nor any correlation of hair Mn content with the biomarker of Fe status. In adults, Montes and associates (2008) observed a negative correlation between blood manganese and hemoglobin levels in persons exposed to a mining and processing plant in Mexico and Baldwin et al (1999) reported an inverse relation between blood Mn and serum Fe. To our knowledge, these relations have not been reported in children. Wasserman et al. (2004; 2006) assessed Hb in their study of neurobehavioral effects of manganese exposure through well water in Bangladeshi children, but they do not report its relation with the biomarkers of Mn.

The relation between mothers' length of residence at childbirth and children's MnH several years later suggests that *in utero* exposure may contribute to higher Mn concentrations in children. Elevated *in utero* exposure may also influence future neurodevelopment. Ericson et al. (2007) measured Mn in the enamel of deciduous teeth, whose formation begins during fetal life. In a prospective study, the authors reported that after adjusting for levels of Pb, children with higher Mn in the uterine phase had higher scores on all scales of disinhibitory behavior. Takser et al. (2003) observed, after adjusting for potential confounders (sex and mother's educational level), negative correlations between Mn levels in the umbilical cord blood and various psycho-motor sub-scales at 3 years (attention, non-verbal memory and manual ability), even though these effects were not observed at 6 years of age.

The findings of this study indicate that children living the vicinity of this Mn alloy production plant have elevated hair Mn levels, which vary with respect to their geo-spatial location of residence. The major sources of Mn are probably fumes expelled by the alloy plant chimneys, dust re-suspension by traffic and possibly dust from the train passing through the village carrying mineral ore and the transformed product. A study of neurobehavioral effects of Mn exposure in these children is currently underway.

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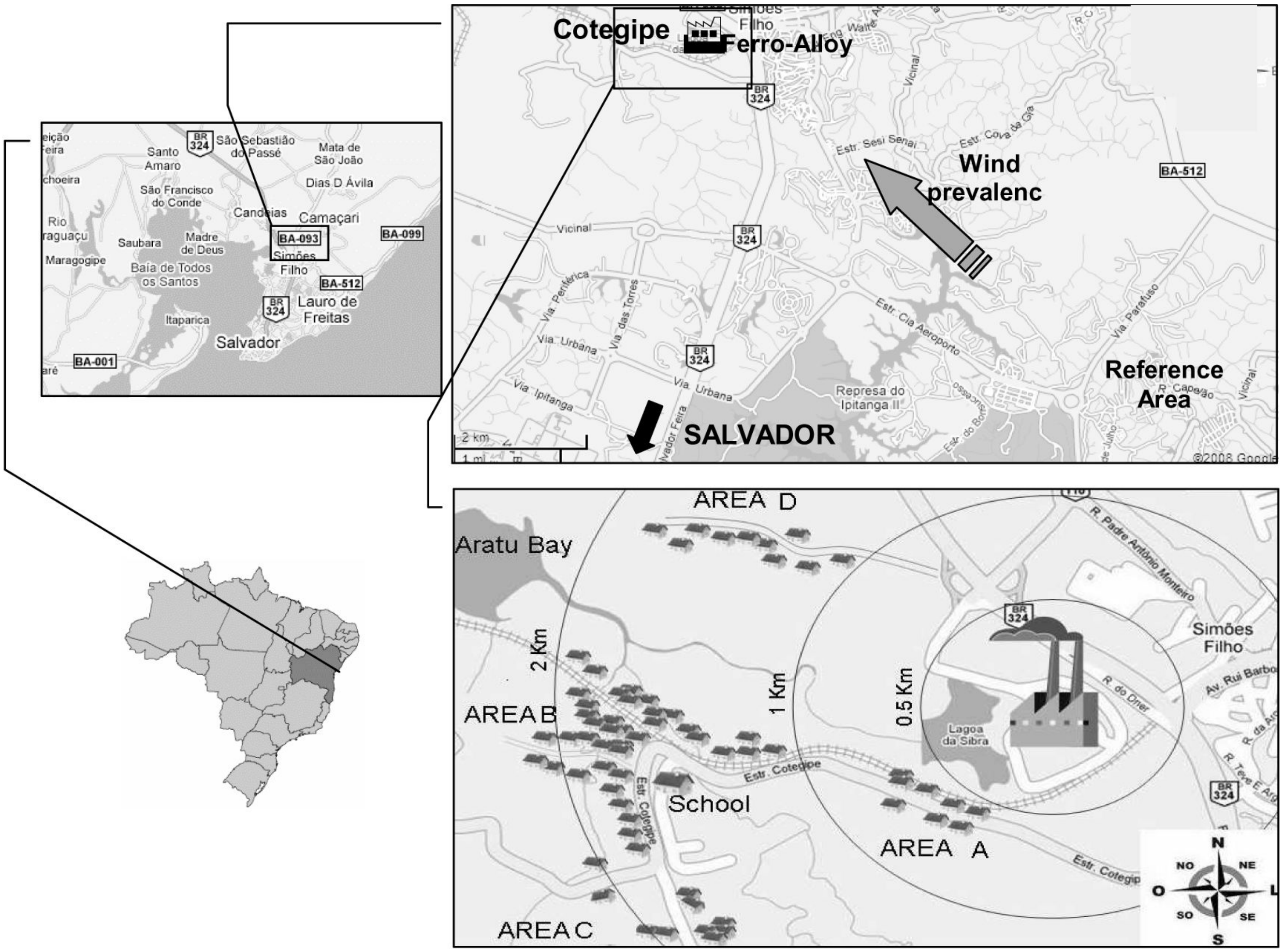

Figure 1.

Schematic map of the Cotegipe Village (exposed community) in Simões Filho town and reference community (Capiarara, Lauro de Freitas), in the Metropolitan Area of Salvador, Bahia, Brazil (top); showing the four residential areas with radial distances from the plant (bottom).

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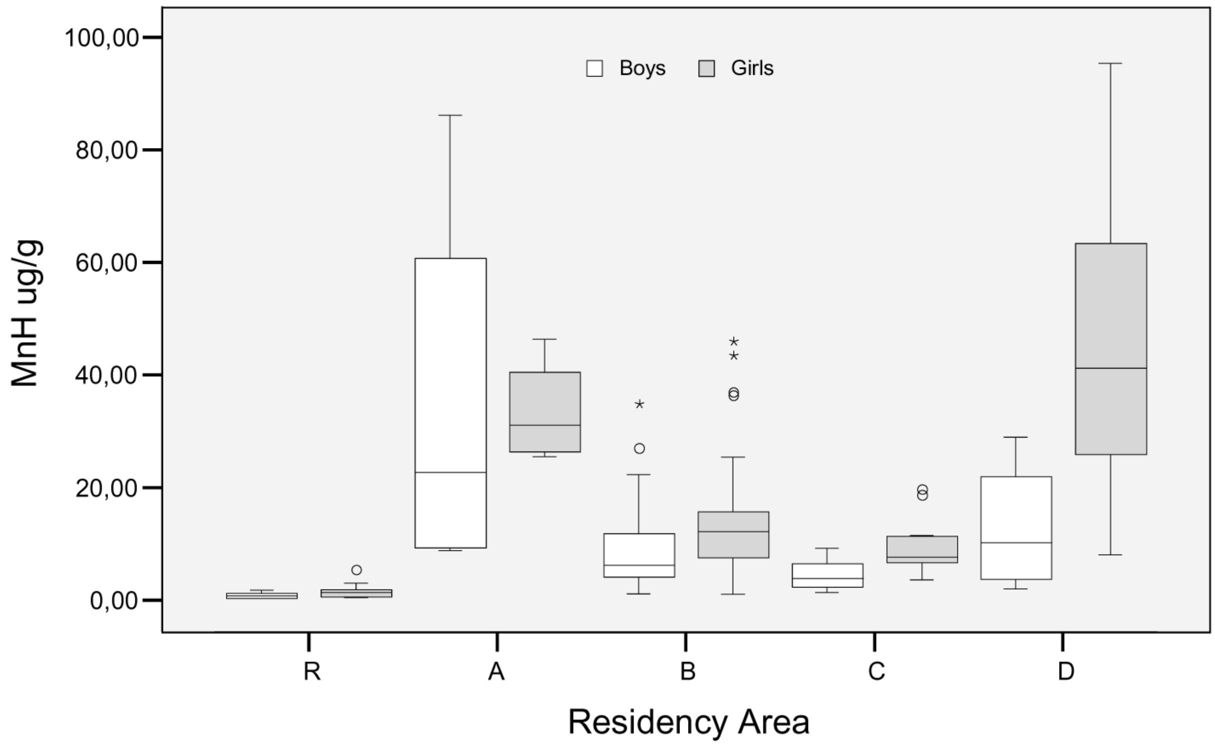

Figure 2.

Box plot of MnH data according to area of residence, clustered by gender. R is for referents and A thru D the four residential areas in the exposed community.

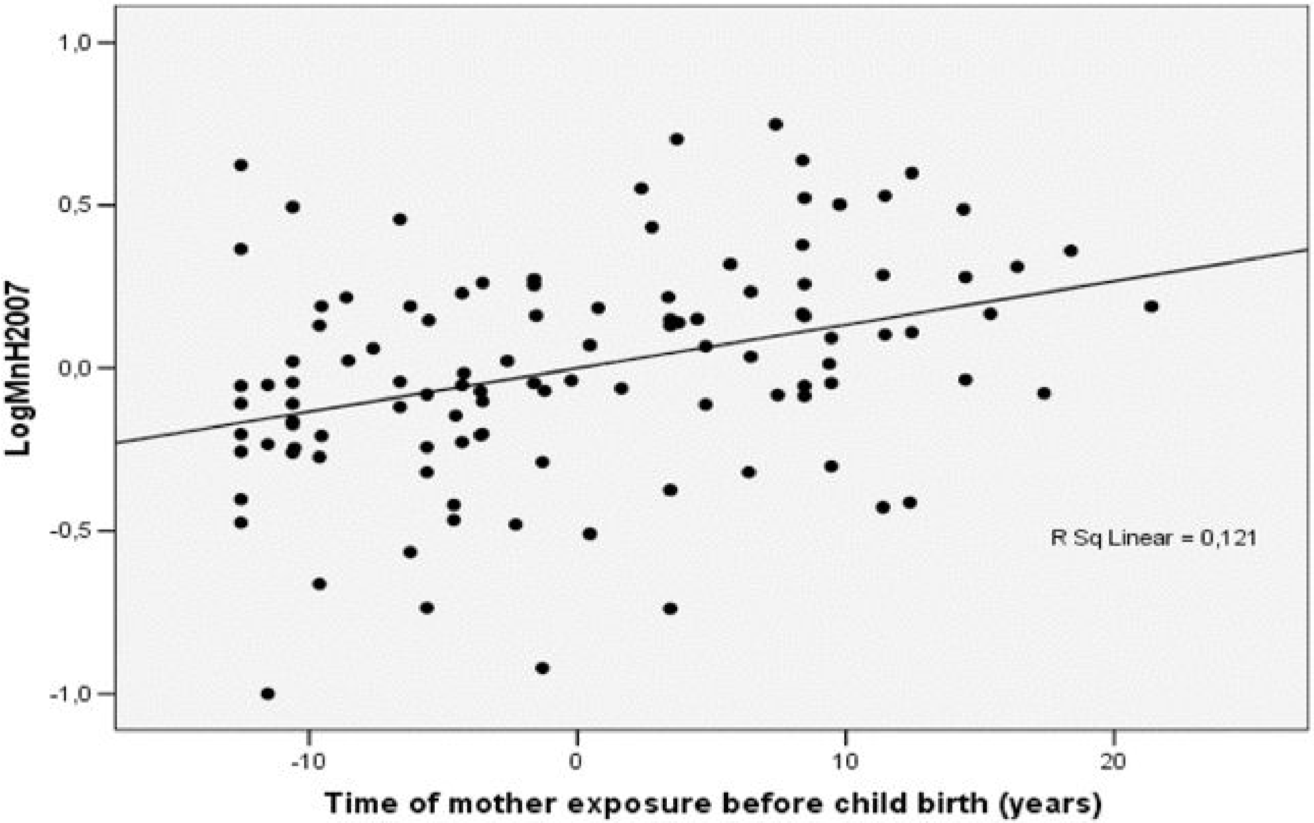

Figure 3.
Residual plot of log transformed Mn concentration in hair *versus* years of maternal exposure time previous to child birth.

Table 1

Summary of socio-demographic characteristics.

	Exposed n (%)					Reference n (%)					P value
Gender											
Boys			53 (48.6%)					19 (44.2%)			n.s.
Girls			56 (51.4%)					24 (55.8%)			n.s.
Ethnicity											
African-Brazilian			79 (72.7%)					35 (79.5%)			n.s.
Non African-Brazilian			30 (27.3%)					8 (20.5%)			n.s
	Mean	**Median**	**SD**	**Min.**	**Max.**	**Mean**	**Median**	**SD**	**Min.**	**Max.**	
Age (months)	78.5	84.9	32.85	10.1	136.5	81.4	83.3	33.73	14.1	132.8	n.s.
Hb (g/dL)	12.3	12.4	1.08	9.7	15.0	12.2	12.1	1.06	9.0	15.0	n.s.
FeS (μg/dL)	57.6	55.0	24.9	10.0	133. 0	52.9	51.5	22.6	14.0	106.0	n.s.
Parents self-reported years of school	2.82	3.0	0.11	1.5	3.6	2.86	3.0	1.0	0.22	4.0	n.s.
Number of years mother lived in the community at the child's birth	10.06	8.0	9.04	0	32.0	8.85	5.0	9.83	0	37.0	n.s.

n.s: not significant for Chi-square or Student's t tests.

Table 2

Hair manganese (µg/g) in children according to area of residency.

Area of residency		n	Geometric mean	Median	SD	Min.	Max.
Reference		43	1.37	1.19	0.95	0.39	8.58
Exposed							
	Area A	8	27.37	31.30	24.50	8.81	86.23
	Area B	75	9.61	9.68	9.61	1.10	46.23
	Area C	15	6.36	6.90	5.42	1.36	19.92
	Area D	11	21.33	28.96	30.38	2.05	95.50

Table 3

Table 3a. Results of the multiple regression model for log MnH with the dependent variables: age, gender and area of residence.

Variable	Unstandardized Coefficients	t Stat.	P value
Intercept	1.506	16.439	<0.001
Age (months)	−0.0004	−0.241	0.810
Gender	−0.255	−3.838	<0.001
Area of residence	−0.206	−4.790	<0.001

Table 3b. Results of the multiple regression model for log MnH with the dependent variables: age, gender, area of residence and maternal exposure time before child's birth (years).

Variable	Unstandardized Coefficients	t Stat.	P value
Intercept	1.915	12.458	<0.001
Age (months)	0.0004	0.210	0.834
Gender	−0.219	−3.508	0.001
Area of residence	−0.502	−6.142	<0.001
Maternal exposure time before child's birth (y)	0.013	3.802	<0.001

logMnH (n=109; r^2=0.268; F=19.420, p<0.001)

logMnH (n=109; r^2=0.368; F=20.378, p<0.001)