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Elevated manganese exposure and school-aged children's behavior: A gender-stratified analysis

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ABSTRACT

High levels of waterborne manganese have been associated with problematic behavior in school-aged children, however to date this has not been reported for children exposed to airborne manganese. The objective of the present study was to examine behavioral traits among children with exposure to airborne manganese from a ferro-manganese alloy plant, located in the metropolitan region of Salvador, Brazil. The study included 34 boys and 36 girls, aged 7–12 years, living in two communities within a 3-km radius from the plant. For each child, hair manganese levels (MnH) and blood lead (PbB) levels were analyzed by graphite furnace atomic absorption spectrometry. The Children's Behavior Check List (CBCL) (Portuguese version validated in Brazil) was administered to parents or caregivers, providing scale scores of internalizing (withdrawn, somatic complaints, and anxious/depressed scales), externalizing (disruptive and aggressive) behaviors and a separate scale for attention problems. Median and range for MnH and PbB were 11.48 μg/g (range: 0.52–55.74); 1.1 μg/dL (range: 0.5–6.1), respectively. Spearman correlation analyses showed that several behavioral indices were significantly correlated with MnH levels for girls, but not for boys: total externalizing behavior (rho = 0.484 vs rho = 0.041) and attention problem scores (rho = 0.542 vs rho = 0.003) coefficients were significantly at $p < 0.001$ level, respectively for girls and boys. No significant correlation was observed with any of the internalizing sub-scales. A linear regression model was fitted with the total externalizing behavior, inattention and total CBCL scores as dependent variables, with log transformed MnH stratified by sex, adjusting for age and maternal IQ. Total externalizing behaviors and attention problem scores were significantly associated with girls' MnH levels but not with boys'. Adjusting for maternal IQ, the β-coefficients for LogMnH associations with total externalizing and attention problems are 8.85 (95%CI 2.44–15.24) and 4.03 (95%CI 1.50–6.56) for girls. For boys, after adjusting for age, the β-coefficients are 0.08 (95%CI 11.51–11.66) and −0.05 (95%CI 4.34–4.25), respectively. The findings of this study suggest a positive association between elevated Mn exposure and externalizing behavioral problems and inattention, with girls presenting more pronounced effects. Future studies on Mn exposure in children should attempt to further elucidate sex and/or gender differences in Mn exposed populations.

1. Introduction

Manganese (Mn) is an essential trace metal, involved in many functions, including bone mineralization, metabolic regulation and protection against oxidative processes (Roth, 2006). It can also be a potent neurotoxin in cases of excessive exposure due to widespread accumulation in the brain (Guilarte et al., 2006). There is an extensive literature dating from 1837 on the neurological and neuropsychological consequences of occupational exposure to Mn among miners and industrial workers (Levy and Nassetta, 2003; Iregren, 1999; Huang et al., 1997; Mergler and Baldwin, 1997). More recently, environmental exposures to air and water-borne Mn have been investigated, particularly among children (for review see: Menezes-Filho et al., 2009; Zoni et al., 2007; Rodríguez-Barranco et al., 2013). Primary school children with elevated Mn exposure consistently display dose-related cognitive deficits (Wasserman et al., 2004, 2006; He et al., 1994; Bouchard et al., 2011; Menezes-Filho et al., 2011; Riojas-Rodríguez et al., 2010; Torres-Agustın et al., 2013). Several studies suggest

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that there may be sex differences, with girls presenting greater effects compared to boys (Roels et al., 2012; Bouchard et al., 2007; Riojas-Rodríguez et al., 2010; Torres-Agustın et al., 2013).

Excess Mn has also been associated with hyperactivity and other behavior disorders in children (Khan et al., 2011; Yousef et al., 2011; Farias et al., 2010; Bouchard et al., 2007; Ericson et al., 2007). Two studies examined children exposed to elevated water-borne Mn. In a community in Quebec, Canada, with high Mn concentrations in their drinking water, Bouchard et al. (2007) reported higher scores on teacher-rated hyperactive and oppositional behavior among children with higher hair Mn, after adjusting for age, sex, and family income. In Bangladesh, Khan et al. (2011) found that children's internalizing and externalizing behaviors, rated by their teachers, were significantly associated with Mn water concentrations, after adjusting for arsenic and demographic co-variables. Other studies have reported associations between Mn and children's behavior patterns, but the possible sources of external Mn exposure were not investigated (Yousef et al., 2011; Farias et al., 2010). Ericson et al. (2007) measured Mn content in shedding teeth. After adjusting for levels of lead in the tooth enamel, children with high levels of Mn had higher scores on all scales of disinhibitory behavior. To date, no study has examined boy' and girls' behaviors in areas where there is elevated airborne Mn.

We have been evaluating children's exposure to manganese from the atmospheric emissions of a ferro-manganese alloy plant in the municipality of Simões Filho, Bahia, Brazil since 2007 (Menezes-Filho et al., 2009, 2011). Air Mn levels in $PM_{2.5}$, the respirable fraction; measured during seven days in 2008 in Cotegipe village presented an average 0.151 $\mu g/m^3$ (range 0.011–0.439 $\mu g/m^3$) (Menezes-Filho et al., 2009). These levels are approximately sevenfold higher than that reported for urban air (0.02 $\mu g/m^3$) (ATSDR, 2012) and vary with distance from the plant and wind direction. Median and range hair Mn levels (MnH) were 9.70 $\mu g/g$ (1.10–95.50 $\mu g/g$). We also reported that cognitive function in school-aged children and their mothers was negatively and significantly associated with Mn hair concentrations (Menezes-Filho et al., 2011).

The present study included children from the community of Cotegipe, as well as the neighboring community of Santa Luzia, Bahia, Brazil. The objective was to examine the association between biomarkers of Mn exposure and their behavior, taking into account relevant co-variables.

2. Materials and methods

The two villages, included in this study, are situated in the municipality of Simões Filho, 30 km from the city of Salvador, State of Bahia, Brazil (Fig. 1). Santa Luzia and Cotegipe are small communities of less than 700 people each. Both communities reside in a 3-km radius from the above-mentioned ferro-manganese alloy plant and mostly in a downwind direction.

2.1. Study design and population

A cross-sectional study design was used. All children aged 7–12 years, who attended the community's elementary schools, were invited to participate. The inclusion criteria were: living in one of the communities for at least five years, being officially enrolled and regularly attending one of the schools. The exclusion criteria were: having an estimated IQ below 68 [which is the low limit for borderline intelligence IQ 70 ± 3.2 (Figueiredo, 2002)], clinical diagnostic of any neurologic problems and hearing disability. Estimated IQ was generated from the sum of the standardized scores of the Vocabulary and Block Design subtests of the Wechsler Intelligence Scale for Children and converted into IQ (Mello et al., 2011).

The school principals provided us with a list of the children enrolled and regularly attending classes. We sent out invitations to the parents or legal guardians of all the children in the specified age range. Those who accepted to participate provided written consent and answered a structured questionnaire upon interview. In order to maintain confidentiality, participating children were properly coded and only the principal investigator and field coordinator had access to the data bank. A total of 95 children from the two communities fulfilled the inclusion criteria. Of these, the parents of 78 children (82%) accepted to participate as volunteers. Three children were excluded, one for having estimated IQ below 68, another had hearing impairment and one had Down's syndrome; five refused to provide biological samples. The final study population was composed of 70 children, 74% of all of the children in the specified age range.

The Simões Filho Education Department gave permission to use the school premises and the community leader gave us full support and provided two rooms in the Santa Luzia Community Center where we carried out the interviews. In Cotegipe village, the local elementary school premises were used. The project was approved by the Federal University of Bahia Ethics Committee.

Questionnaires: Trained interviewers applied structured questionnaires to collect socio-economic and life style information of the volunteered families. The socioeconomic classification from the Brazilian Association of Population Studies (ABEP, 2011), based on the possessions of comfort items in the home (washing machine, fridge and vacuum cleaner, car, color TV, bathroom, etc.) was applied. The total ABEP score is ranked into five descending classes (from A to E).

Anthropometry: The same person performed all weight and height measurements. Children took off their shoes but kept their clothes for both assessments. Weight was measured using an upright scale (Urano UP150S), with a capacity to weigh 150 kg in 100-g increments. Height was taken using a measuring board. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters. Height-for-age (HA) *z*-score was calculated using the AnthroPlus software (WHO, 2009) based on the WHO reference 2007 for 5–19 years.

Hair measurements: A tuft of hair of approximate 0.5 cm diameter was cut off as close as possible to the scalp in the occipital region, with a surgical stainless steel scissor and stored in plastic bag until processing. Detailed information on hair sampling, washing procedure and Mn determination by GFAAS are reported elsewhere (Menezes-Filho et al., 2009). 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 2 h on a 90 °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 (Human hair from International Atomic Energy Agency, IAEA-085) were analyzed using electrothermal atomic spectroscopy with Zeeman background correction (GTA-120, Varian Inc.). The intra-batch and batch-to-batch precisions were 2.36% and 5.90%. All samples and reference materials were analyzed in duplicates and a difference of 10% or less was considered acceptable.

Blood measurements: Children's venous blood samples were collected from the cubital vein into sodium–EDTA vacuum tubes proper for metal analysis (Vacutainer, Bencton Dickinson, USA). Lead (Pb) is an ubiquitous contaminant and a recognized neurotoxin, associated with effects on cognition and behavior in children at low blood Pb (PbB). PbB concentration was also

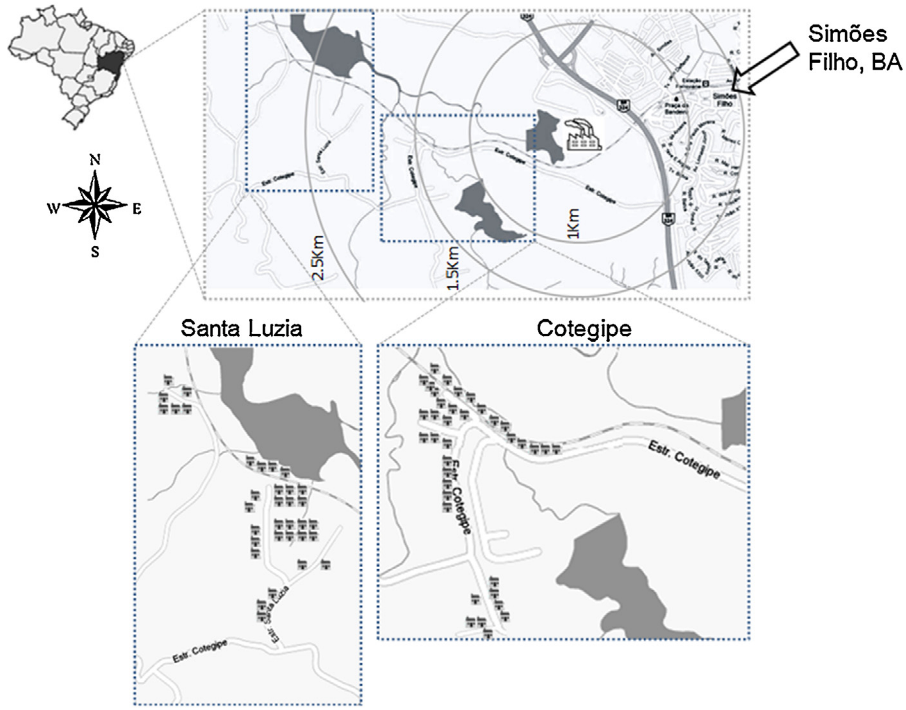

Fig. 1. Schematic map of the studied area, showing distances of the communities to the ferro-manganese alloy plant in the municipality of Simões Filho, Bahia, Brazil.

measured by GFAAS with Zeeman background correction (GTA-120, Varian Inc.) according to the methods described by WHO (1996). For quality control, Lyphocheck® whole blood metals controls, levels 1 and 2 (BioRad, France) were analyzed with each batch. The detection limit was set to 0.5 μg/dL, intra and inter-precision were 4.5% and 8.2%, respectively. The accuracy was always in the 94–105% range.

2.2. Measures of behavior

The Child Behavior Check List (CBCL) Portuguese version, validated in Brazil (Bordin et al., 1995), was administered to parents or caregivers. This questionnaire assesses a broad range of behavioral and emotional problems; reliability and validity are well-established. The test provides a standard total problems score, two broad band factors: (i) externalizing, reflecting aggressive, defiant, disobedient, and destructive behavior, and (ii) internalizing, reflecting anxious, fearful, depressed, and withdrawn behavior, and (iii) a narrow band factor for attention problems.

The CBCL was individually applied by a trained psychologist at an interview with the main caregiver in a quiet room. The respondent was required to grade the child's behaviors as 2, if it happened very often or if it was very likely; 1 if it was less likely to happen; and 0 if it was not true at all. Whenever a statement was not well understood some examples were given.

Maternal or main caregiver intelligence was estimated using the Vocabulary and Block Design subtests from the Wechsler Adult Intelligence Scale (WAIS) 3rd version, standardized for Brazil (Nascimento, 2005). They were also asked to provide an occipital hair sample, which was collected with the same procedure described above for the children.

2.3. Statistical analyses

Descriptive statistics were used to examine the distribution of socio-demographic information, bioindicators of Mn and Pb exposure and CBCL scores. For continuous variables, the arithmetic mean (AM) was used, standard deviation, median, minimal and maximum are presented. Since previous studies suggest possible differences in boys' and girls' response to Mn exposure (Roels et al., 2012), we present total group and sex-stratified results.

Spearman's correlation analysis was applied to evaluate associations between co-variables and exposure biomarkers, as well with behavioral outcomes. In addition, children's MnH level were divided into tertiles, with the cut-off values 7.74 μg/g and 15.91 μg/g, labeled Low, Medium and High. One-way analysis of variance was used to test the hypothesis of differences among the strata and Dunnett T3 post hoc test to check in group differences.

The distributions of hair and blood metal levels were skewed and data were $\log_{10}$ transformed for further analyses. Multiple linear regression analysis was used to examine the factors that were associated to behavior indices and attention problems in boys and girls. In addition, to LogMnH, the variables that were shown to be significantly associated with the outcomes in the stratified correlation analyses were entered into the models. The significance level of $p = 0.05$ was used and residual analyses were performed to verify the model's parameters. All statistical analyses were performed using SPSS version 17 software.

3. Results

3.1. Population characteristics and exposure

Data in Table 1 presents socio-demographic characteristics, anthropometric indices, metal biomarker levels and behavior indices for boys and girls, as well as major characteristics of the main caregivers. The relative frequency of boy and girls was almost the same (51% girls). The majority (52.9%) was raised in a structured family (both parents living together), and 38.6% lived only with their mother. Sixty-one respondents (87%) were mothers, the rest were grandmothers (9%) and aunts or step-mothers. The majority (82.2%) of the mothers, when pregnant, lived in the communities and their children lived in the

Table 1
Socio-demographic characteristics, metal exposure biomarkers and behavioral indices of the studied population according to children's sex.

	Boys ($n = 34$)					Girls ($n = 36$)				
	Mean	SD	Median	Min.	Max.	Mean	SD	Median	Min.	Max.
Children										
Age (years)	9.5	1.6	10.0	7	12	9.4	1.6	10.0	7	12
z-Score HA	−0.11	0.86	−0.18	−1.21	2.38	−0.17	1.31	0.00	−3.18	2.60
Head circumference (cm)	52.9	1.3	53.0	49.5	56.1	52.6	2.0	53.0	48.2	58.5
Blood lead (μg/dL)	1.2	1.3	0.5	0.5	6.1	1.1	0.9	0.5	0.5	4.0
Hair Mn (μg/g)	15.3	9.9	12.1	1.5	48.5	13.9	13.4	12.4	0.5	55.7
Externalizing behaviors										
Disruptive	3.5	3.4	3.0	0	14	2.9	2.8	2.0	0	12
Aggressiveness	9.3	5.6	9.0	0	23	9.4	7.6	8.0	0	28
Total Externalizing	12.8	8.5	12.0	0	37	12.3	9.76	10.0	0	40
Internalizing behaviors										
Anxiety/depression	4.3	3.2	3.0	0	12	5.4	3.7	5.0	0	16
Withdrawn	2.7	2.3	2.0	0	10	2.7	2.2	2.0	0	7
Total internalizing	7.0	5.5	5.0	0	22	8.1	5.9	7.0	0	23
Inattention	6.1	3.5	6.0	0	13	4.9	3.9	5.0	0	13
Total CBCL score	35.0	17.6	32.0	2	78.0	34.3	19.1	32.0	2	92
Main caregiver										
Mothers	61	87%								
Grandmothers	6	9%								
Others	3	4%								
Age (years)	35.9	2.1	112	16	62					
Hair Mn (μg/g)	10.71	10.34	7.29	0.62	44.61					
Estimated IQ	86.7	10.9	83	71	117					
Total ABEP score	13.9	3.79	14	7	27					

communities since they were born. The socio-economic status of the families ranked as class "C" (54%) and "D" (39%); very few were either "E" or "B" (3%) and none classified as "A". A substantial portion of the families received support from the Brazilian government's social program against poverty. On average, mothers or caregivers had seven years of formal schooling. Their estimated IQ (IQ = 86.7) is on average one standard deviation lower than to what is expected for adults of their age. There were no statistical differences in any of the total or on the sub-scales of the behavior outcomes according to sex.

Children's average age was 9.4 (SD ±1.6) years, with no significant difference between boys and girls. There was no statistical difference in their chronic nutritional index (height for age *z*-score). On average there was a tendency for malnourishment. One girl presented HA *z*-score of −3.18, indicative of stunting.

Blood lead levels among these children were particularly low, even when compared with the current attention value of PbB of 5 μg/dL (CDC, 2012). On average, it was 1.2 ± 0.9 μg/dL, only one boy had PbB above the attention value.

The median and range of Mn hair levels were similar for boys and girls: 12.1 μg/g (1.5–48.5) and 12.4 μg/g (0.5–55.7), respectively. Mothers or caregivers' MnH levels were equally high, median and range were 7.22 μg/g (0.62–44.61). Maternal and children's hair Mn were positively correlated, but did not reach the significance cut-off (rho = 0.220, $p = 0.086$) (Table 2).

Table 2
Spearman correlation coefficients for metal biomarkers, socio-demographics and neurobehavioral outcomes for boys ($n = 34$) and girls ($n = 36$).

		MnH	PbB	HA *z*-score	Age	Maternal MnH	Maternal IQ	Total ABEP	Disrupt. score	Aggres. score	Total externalizing	Total internalizing	Inattention score
Childrens'MnH	Boys	1.000	−0.065	−0.359*	0.511**	0.191	−0.407*	−0.051	0.227	0.041	0.071	0.073	0.003
	Girls	1.000	0.104	−0.199	−0.054	0.233	−0.455**	−0.193	0.391*	0.480**	0.484**	0.135	0.542**
Children's PbB	Boys		1.000	−0.022	−0.242	0.312	−0.442*	−0.359	−0.129	−0.285	−0.245	−0.180	−0.134
	Girls		1.000	−0.123	0.269	−0.110	−0.237	−0.091	0.189	0.189	0.210	0.084	0.193
Children's HA *z*-score	Boys			1.000	−0.446**	0.023	0.455**	0.070	−0.140	−0.054	−0.091	0.158	−0.131
	Girls			1.000	−0.438**	0.122	0.056	0.091	−0.430**	−0.038	−0.131	0.068	0.011
Child. Age	Boys				1.000	−0.216	−0.064	0.006	0.451**	0.401*	0.450**	0.097	0.309
	Girls				1.000	−0.131	0.025	−0.043	0.035	−0.050	−0.018	0.061	−0.084
Mater. MnH	Boys					1.000	−0.361*	−0.355*	−0.135	−0.186	−0.166	−0.080	−0.082
	Girls					1.000	−0.279	−0.291	0.048	0.125	0.139	−0.108	0.096
Maternal IQ	Boys						1.000	0.367*	−0.210	0.019	−0.059	−0.096	−0.328*
	Girls						1.000	0.504**	−0.185	−0.357*	−0.356*	−0.183	−0.350*
Total ABEB	Boys							1.000	−0.041	0.073	0.050	−0.080	−0.220
	Girls							1.000	−0.197	−0.318	−0.322	−0.108	−0.221
Disruptive score	Boys								1.000	0.632**	0.794**	0.260	0.504**
	Girls								1.000	0.580**	0.742**	0.182	0.608**
Aggressiveness score	Boys									1.000	0.963**	0.236	0.430**
	Girls									1.000	0.970**	0.391*	0.828**
Total externalizing	Boys										1.000	0.238	0.454**
	Girls										1.000	0.376*	0.846**
Total internalizing	Boys											1.000	0.481**
	Girls											1.000	0.465**
Inattention score	Boys												1.000
	Girls												1.000

* $p < 0.05$.
** $p < 0.001$.

Table 2 presents the Spearman correlation matrix for the relevant variables stratified by sex. Children's MnH and PbB levels were inversely correlated with maternal IQ: for boys rho = −0.407 ($p < 0.001$) and rho = −0.442 ($p < 0.05$), respectively, and for girls: rho = −0.455 ($p < 0.001$) and rho = 0.237 ($p = 0.06$) for girls, respectively. Mothers' and caregivers' MnH was likewise negatively correlated with their intellectual performance (rho = −0.328, $p = 0.007$). The chronic nutritional index was inversely correlated with MnH levels, but statistical significance was observed only for boys (rho = −0.359, $p < 0.05$). The social-economic index (total ABEB score) was positively correlated with maternal IQ (rho = 0.452, $p < 0.001$) and inversely with her MnH levels (rho = −0.301, $p = 0.016$).

3.2. Children's Behavioral Indices

Externalizing behavior outcomes were significantly and directly correlated with girl's MnH levels: disruptive (rho = 0.391, $p < 0.05$), aggressive (rho = 0.480, $p < 0.001$) and total externalizing (rho = 0.484, $p < 0.001$) behaviors. For boys, correlation coefficients were not significantly correlated. The same pattern was observed with attention problem scores; for girls it was significantly correlated with their MnH levels (rho = 0.542, $p < 0.001$), but for boys no correlation was observed (rho = 0.003). On the other hand, no association was observed with any of the internalizing behaviors for either girls or boys. Age was positively correlated with boys' disruptive scores (rho = 0.451, $p < 0.001$), aggressive score (rho = 0.401, $p < 0.05$), total externalizing behavior (rho = 0.450, $p < 0.001$) and attention problem (rho = 0.309), but the latter did not reach significance level. No correlation was observed between girls' age and any of the behavior indices. Children's attention problem scores were negatively correlated with maternal IQ, rho = −0.328 ($p < 0.05$) for boys and rho = −0.350 ($p < 0.05$) for girls. Total ABEP scores were negatively correlated with the behavioral indices and attention problem scores, but did not reach statistical significance.

Fig. 2 shows the box-plot graphs of the total internalizing and externalizing behaviors and attention problem scores for boys and girls for MnH tertiles. No inter-tertile difference was observed for boys. The F statistics for one-way analysis of variance were: 0.084, $p = 0.920$ (internalizing score); 1.717, $p = 0.197$ (externalizing score) and 0.429, $p = 0.256$ (attention problems). For girls, no differences were observed for the total internalizing behavior scores (F statistic = 0.312, $p = 0.732$), but there were significant inter-tertile differences for the total externalizing behavior scores (F statistic = 5.263, $p = 0.011$). The Dunnett T3 post hoc test, once non homogenous variance was assumed, detected that girls in the highest MnH level tertile had mean externalizing score with a borderline difference ($p = 0.065$) from those in the lowest MnH levels. For girls, the attention problem scores were also statistically different according MnH strata (F statistic = 7.030, $p = 0.003$). The post hoc test indicated that girls in the medium and high MnH tertiles had statistically different attention problem scores when compared with girls in the lowest tertile, while the attention problem scores in the medium MnH level tertile did not differ from those in the high tertile.

Table 3 shows the linear regression models for total CBCL score, total externalizing behavior and attention problem scores associations with LogMnH levels, either adjusted for age or maternal IQ for boys and girls, separately. Total CBCL scores, externalizing behavior and attention problem scores were significantly associated with LogMnH for girls, but not for boys. Girls' total externalizing behavior and inattention, independently of maternal IQ was significantly associated with LogMnH: β-coefficient = 8.85 (CI95% 2.44–15.25) and 4.03 (CI95% 1.50–6.56), respectively. Total ABEP scores and HA z-score were entered into the models but the associations with LogMnH remained the same. When all participants are included without stratifying for sex, LogMnH levels are still significantly associated with total externalizing behavior and attention problem scores with non-standardized β-coefficients of 7.57 (IC95% 2.14–13.00) and 2.97 (IC95% 0.78–5.17), respectively, taking into account child's sex, age and maternal IQ. Fig. 3a presents the scattergrams for adjusted outcomes (total CBCL score, total externalizing behavior and attention problem scores) vs. LogMnH levels with the linear regression and confidence intervals of the slope for these measures for boys and girls, separately. Fig. 3b shows the scattergram for the adjusted model of total CBCL score vs. LogMnH, adjusted covariates are sex, age and maternal IQ.

4. Discussion

The findings of this study suggest that children's elevated exposure to airborne manganese is positively and significantly associated with externalizing behaviors and attention problems, particularly for girls, despite similar hair Mn concentrations in boys and girls.

Hair Mn concentrations in both communities are high and corroborate our previous report on the Cotegipe community exposure in 2008 (Menezes-Filho et al., 2011), in which MnH levels ranged 0.1–86.7 μg/g. MnH levels in these communities are, on average, fifteen times higher than the levels reported for the general Brazilian population (0.25–1.15 μg/g) (Miekeley et al.,

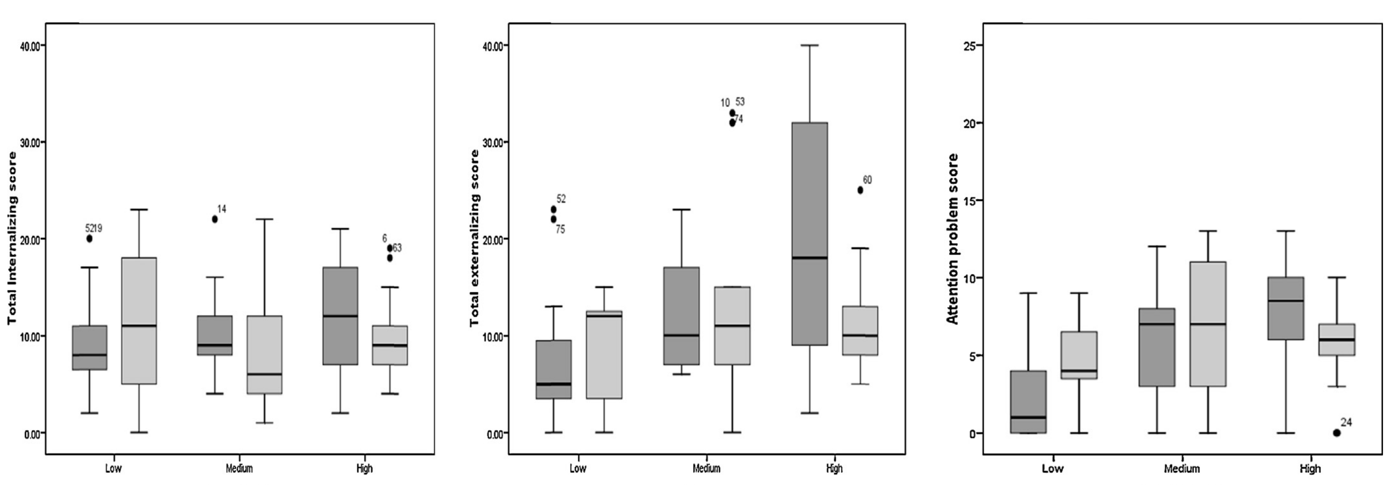

Fig. 2. Total internalizing and externalizing behavior and attention problem scores of boys and girls according to MnH level tertiles.

Table 3
Linear regression models for total externalizing behavior and attention problem scores stratified and non-stratified by sex.

			Non-standardized β-coefficients	p-Value	IC95%
Sex stratified models					
Total CBCL score					
	Boys	Intercept	12.00	0.518	−25.50 to 49.50
		LogMnH	1.98	0.869	−22.23 to 26.18
		Age	0.16	0.374	−0.21 to 0.54
	Girls	Intercept	44.06	0.138	−14.87 to 96
		LogMnH	16.18	0.014	3.45–28.91
		Maternal IQ	−0.29	0.358	−0.92 to 0.34
Total externalizing					
	Boys	Intercept	−2.33	0.793	−20.28 to 15.63
		LogMnH	0.08	0.989	−11.51 to 11.67
		Age	0.12	0.180	−0.06 to 0.29
	Girls	Intercept	16.17	0.275	−13.48 to 45.81
		LogMnH	8.85	0.008	2.44–15.25
		Maternal IQ	−0.14	0.374	−0.45 to 0.18
ion problems					
	Boys	Intercept	11.90	0.092	−2.05 to 25.86
		LogMnH	−0.05	0.982	−4.34 to 4.25
		Maternal IQ	−0.07	0.314	−0.21 to 0.07
	Girls	Intercept	5.49	0.347	−6.22 to 17.20
		LogMnH	4.03	0.003	1.50–6.56
		Maternal IQ	−0.05	0.420	−0.18 to 0.08
Non-stratified models					
Total CBCL score		Intercept	46.06	0.087	−6.84 to 98.95
		LogMnH	13.99	0.013	3.06–24.94
		Sex	−3.40	0.434	−12.05 to 5.24
		Age	−0.03	0.809	−0.25 to 0.194
		Maternal IQ	−0.21	0.357	−0.67 to 0.24
Total externalizing		Intercept	14.98	0.259	−11.28 to 41.25
		LogMnH	7.57	0.007	2.14–13.00
		Sex	−1.85	0.392	−6.15 to 2.44
		Age	0.02	0.968	−0.11 to 0.11
		Maternal IQ	−0.09	0.409	−0.32 to 0.13
Attention problems		Intercept	5.73	0.286	−4.91 to 16.37
		LogMnH	2.97	0.009	0.78–5.17
		Sex	0.53	0.552	−1.22 to 2.26
		Age	0.01	0.895	−0.04 to 0.05
		Maternal IQ	−0.05	0.266	−0.14 to 0.04

1998). The correlation between mother's and children's MnH probably derives from similar home exposures. The MnH concentrations observed here are considerably higher than those reported in other studies of Mn exposed children (Bouchard et al., 2011; Riojas-Rodríguez et al., 2010; Wright et al., 2006); 76.8% are above the 3.0 μg/g cut-off value, which Bouchard et al. (2007) used to test the association with hyperactive behaviors.

Although hair Mn is ideal for use with children since it is non-invasive, there may be external contamination from airborne Mn depositing on hair (Eastman et al., 2013). If this were the case, hair Mn concentrations may be higher, but the associations would be similar. The negative correlation between nutritional status (HA z-score) and MnH levels was similar to our previous findings (Menezes-Filho et al., 2011). Long term deficiency in protein and possibly in iron may favor a higher absorption of Mn from diet and perhaps more brain accumulation and possibly more excretion through hair.

The CBCL has been used in two other studies of Mn exposure, with similar results; one used mother's assessment (Ericson et al., 2007), while the other used teacher assessment (Khan et al., 2011). However, contrary to the present study, these authors observed positive associations with both externalizing and internalizing behaviors, while in our study, internalizing behavior was not associated to hair Mn levels. A possible explanation for the observed association between aggressive and defiant behaviors and Mn exposure may be decreased serotonin, which is responsible for the inhibition control. In a study of Chinese school-aged children exposed to Mn in drinking water, Zhang et al. (1995) reported that serum neurotransmitters, especially serotonin, were positively correlated with overall school average score ($r = 0.635$) and their levels were markedly lower in exposed compared to non-exposed children ($p < 0.01$). Clarke et al. (1999) reported an inverse relation between externalizing behavior score on the CBCL at 30 months of age and 5-hydroxyindoleacetic acid (5-HIAA), a main metabolite of serotonin, measured in cerebrospinal fluid (CSF) just after birth. Low CSF 5-HIAA was likewise reported in children and adolescents with aggressive and disruptive behavior (Kruesi et al., 1992).

Since several studies have shown sex differences in Mn exposed children (Torres-Agustın et al., 2013; Roels et al., 2012; Bouchard et al., 2007; Riojas-Rodríguez et al., 2010), in the present study, we examined boys and girls separately. Contrary to Khan et al. (2011), who reported that teacher-rated externalizing behavior scores were higher in boys than in girls, in this study, parent-rated behaviors were similar for boys and girls. However, only girls showed significant associations between MnH levels and externalizing behavior and attention problem scores. Several factors may explain the differences between boys and girls. First, this may reflect a gender difference in socialization and the way in which parents view boys' and girls' behavior. Aggressive and disruptive behaviors and problems of attention may be considered more 'normal' in younger boys than in younger girls. Interestingly, contrary to girls, boys' attention problems increase with age. It is also possible that because of the small numbers, we were unable to

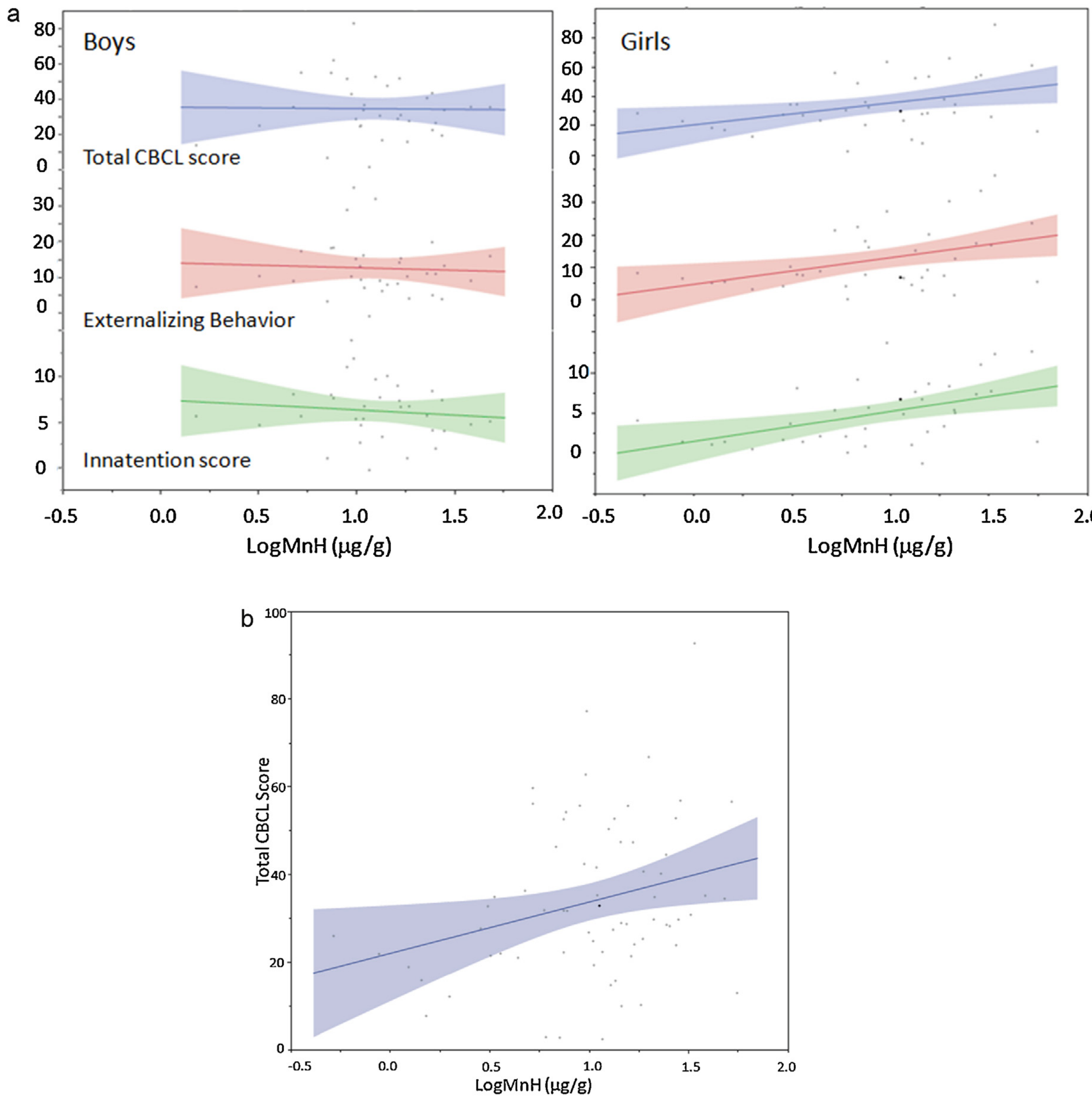

Fig. 3. (a) Scattergrams of adjusted total CBCL, total externalizing behavior and Inattention scores vs. log of MnH levels. Boys' scores were adjusted for age and girls' for maternal IQ. (b) Scattergrams of adjusted total CBCL scores vs. log of MnH levels adjusted for sex, age and maternal IQ.

detect an effect of Mn exposure in boys. An alternative hypothesis would be biological differences in response to Mn. Madison et al. (2011) reported sex differences in striatal morphology, with females demonstrating larger effects than males despite similar concentrations of Mn. More mechanistic studies that differentiate males and females are needed to elucidate possible biological differences.

Lead is well known neurotoxin that has been associated with hyperactive and attention deficit in children (WHO, 2006). We have previously demonstrated in the same communities studied here that Pb exposure is higher in children from families who reported burning domestic waste and having at least one smoker at home (Menezes-Filho et al., 2012). In the current study, PbB levels were below the attention value of 5 μg/dL, and no effect on attention or any behavioral indices was observed. PbB concentration did vary with the socio-economic status index, although the difference was not significant. Poorer children may be more exposed to environmental contaminants through diet, water or living conditions (Bellinger, 2009).

Our study has several limitations. The most important is the small number of children, due to size of the communities and the number of eligible school children, which limited our statistic analysis, particularly when stratifying for sex. There is also the intrinsic limitation of a cross-sectional design due the temporality issue. More than 80% of the children were conceived and lived in the community since birth and we cannot distinguish between pre and post-natal effects. There were 61 mothers and 85% of them carried out their pregnancies in the communities. Despite the limited size of our study population, we had similar frequency of boys and girls and we were also able to measure maternal exposure and cognition. It is noteworthy that mothers and caretakers likewise had elevated Mn, which was associated both to their IQ and to socio-economic status.

To our knowledge, this study is the first to suggest that excessive airborne Mn exposure may have detrimental effects on children's externalizing behavior and is positively associated with attention problems. These findings need to be further investigated in a prospective study.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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