



Verbal Memory and Learning in Schoolchildren Exposed to Manganese in Mexico

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Abstract

Manganese (Mn) is an essential nutrient for cellular function, but in high concentrations, it is neurotoxic. Environmental exposure to Mn has been associated with cognitive effects in children. This study aimed to assess the effect of environmental exposure to Mn on verbal memory and learning in schoolchildren residents from two municipalities in the state of Hidalgo, Mexico. Cross-sectional studies were conducted in 2006 and 2013 with a total of 265 schoolchildren of 7 to 11 years old. *Children's Auditory Verbal Learning Test-2* (CAVLT-2) was used to assess verbal memory and learning. Mn exposure tertiles were defined according to hair manganese (MnH) levels determined by atomic absorption spectrophotometry. Linear regression models were used to estimate the association between MnH levels and CAVLT-2 scores. The models were adjusted by potential confounders. The lowest and highest exposure tertiles were defined below and above MnH levels of ≤ 0.72 and ≥ 3.96 $\mu\text{g/g}$, respectively. Mn exposure was significantly associated with an average of 5- to 9-point decrease in learning curves and summary CAVLT-2 scores in the highest tertile. This study adds to the evidence of decreased verbal memory and learning in schoolchildren environmentally exposed to manganese.

Keywords Hair manganese · Mining area · Verbal memory · Verbal learning · Schoolchildren

Highlights

- Measured Mn in hair.
- Assessed the verbal memory and learning in schoolchildren.
- A negative association between MnH and verbal memory and learning performance in schoolchildren.

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Abbreviations

Mn	Manganese
GABA	Gamma-aminobutyric acid
CNS	Central Nervous System
CAVLT-2	Children's Auditory Verbal Learning Test-2
MnH	Hair manganese
μg/g	Micrograms per gram
Pb	Lead
PbB	Blood lead
μg/dL	Micrograms per deciliter
Hb	Hemoglobin
g/dL	Grams per deciliter

Introduction

Manganese (Mn) is a transition metal that plays an essential role in the enzymatic reactions necessary for the cellular and physiological functioning of humans; however, in high concentrations, it can be neurotoxic (Saric and Lucchini 2007; Jiménez and Kuhn 2009; ATSDR 2012). Mn is present in water, soil, air, and food in a natural and anthropogenic form (ATSDR 2012). There are different routes of exposure to Mn; however, inhalation is considered a direct route to transfer Mn to the brain by environmental exposure (Carvalho et al. 2014).

The process of transporting Mn to the human brain has not yet been fully described. Studies in animal models suggest that after Mn has been inhaled, it can reach the brain through the olfactory bulb (Brenneman et al. 2000; Dorman et al. 2002; Elder et al. 2006; Saputra et al. 2016). The Mn continues to move through the synaptic connections, affecting mainly the functioning of glia (Aschner et al. 2002; Zuleta 2007). Several studies have shown that exposure to Mn causes nitrosative stress in glia and neurons of the basal ganglia causing its dysfunction (González-Scarano and Baltuch 1999; Moreno et al. 2009).

It has been identified that Mn accumulates and generates toxicity in the basal ganglia and substantia nigra (Dobson et al. 2004; Bowman et al. 2011). The basal ganglia produce gamma-aminobutyric acid (GABA), glutamate, and dopamine neurotransmitters modulate the speed of the nerve impulse between the neurons and also participate in cognitive functions such as memory and learning (Zuleta 2007). Children are considered susceptible to the neurotoxic effects from heavy metals and other environmental pollutants, because their central nervous system (CNS) is in different stages of development and maturation, and can be influenced on the performances of specific areas of the brain (Rice and Barone 2000; Weiss 2000; Winder 2010).

Nowadays, there is epidemiological evidence about the effect of Mn on the neurocognition of schoolchildren. The neurocognitive effects include attention problems (Menezes-Filho et al. 2014; Oulhote et al. 2014a; Shin et al. 2015), motor

function impairment (Hernández-Bonilla et al. 2011; Lucchini et al. 2012; Rugless et al. 2014), decreased verbal memory and learning (Wright et al. 2006; Carvalho et al. 2018), alterations of visuoception, visuospatial ability and visual memory (Hernández-Bonilla et al. 2016; Bauer et al. 2017), poor language development (Khan et al. 2011; Rink et al. 2014), executive function and working memory deficits (Carvalho et al. 2014; Bauer et al. 2017; Carvalho et al. 2018), and poor intellectual performances (Wright et al. 2006; Bouchard et al. 2011; Riojas-Rodríguez et al. 2010; Menezes-Filho et al. 2011; Carvalho et al. 2014; Wasserman et al. 2015).

The aim of this study was to assess the association of environmental exposure to Mn on verbal memory and learning in schoolchildren from 7 to 11 years old. The study was performed in two municipalities from the state of Hidalgo in Mexico, with different distances to one of the biggest Mn deposits in the world, with extraction and processing activities underway.

Materials and Methods

Study Design and Population

Two cross-sectional studies were conducted in children aged 7 to 11 years old (2006 and 2013). The schoolchildren environmentally exposed to Mn were living in the rural communities of Chiconcoac and Tolago into the municipality of Lolotla, mining district of Molango, Hidalgo. The district has a world-class Mn deposit, with 30.2 million tons of this element for an estimated production of 39 years and 250 million tons of potential reserves for the next 200 years (Fig. 1).

Children from the non-mining area were residents of the rural municipality of Agua Blanca, Hidalgo, located 80 km away from the mining district of Molango. These children were considered as non-environmentally exposed to Mn because there were no known sources of environmental exposure to this metal in the area. Children from the non-mining area were selected considering similar socioeconomic conditions to those from the mining area according to the Margination Index of the National Population Council (CONAPO 2005).

In all communities, children and their parents were invited to participate voluntarily through informative meetings. Selected participants were children aged 7 to 11 years old, attending elementary school, and with a minimum residence of 5 years in their community. Schoolchildren with a neurological or psychiatric problem or any disability that interfered with the neuropsychological tests' execution were excluded from the study. A total of 265 (138 girls and 127 boys) were evaluated: 155 in 2006 and 110 in 2013; 144 from the municipality of Lolotla (72 in 2006 and 72 in 2013) and 121 from the municipality of Agua Blanca (83 in 2006 and 38 in 2013).

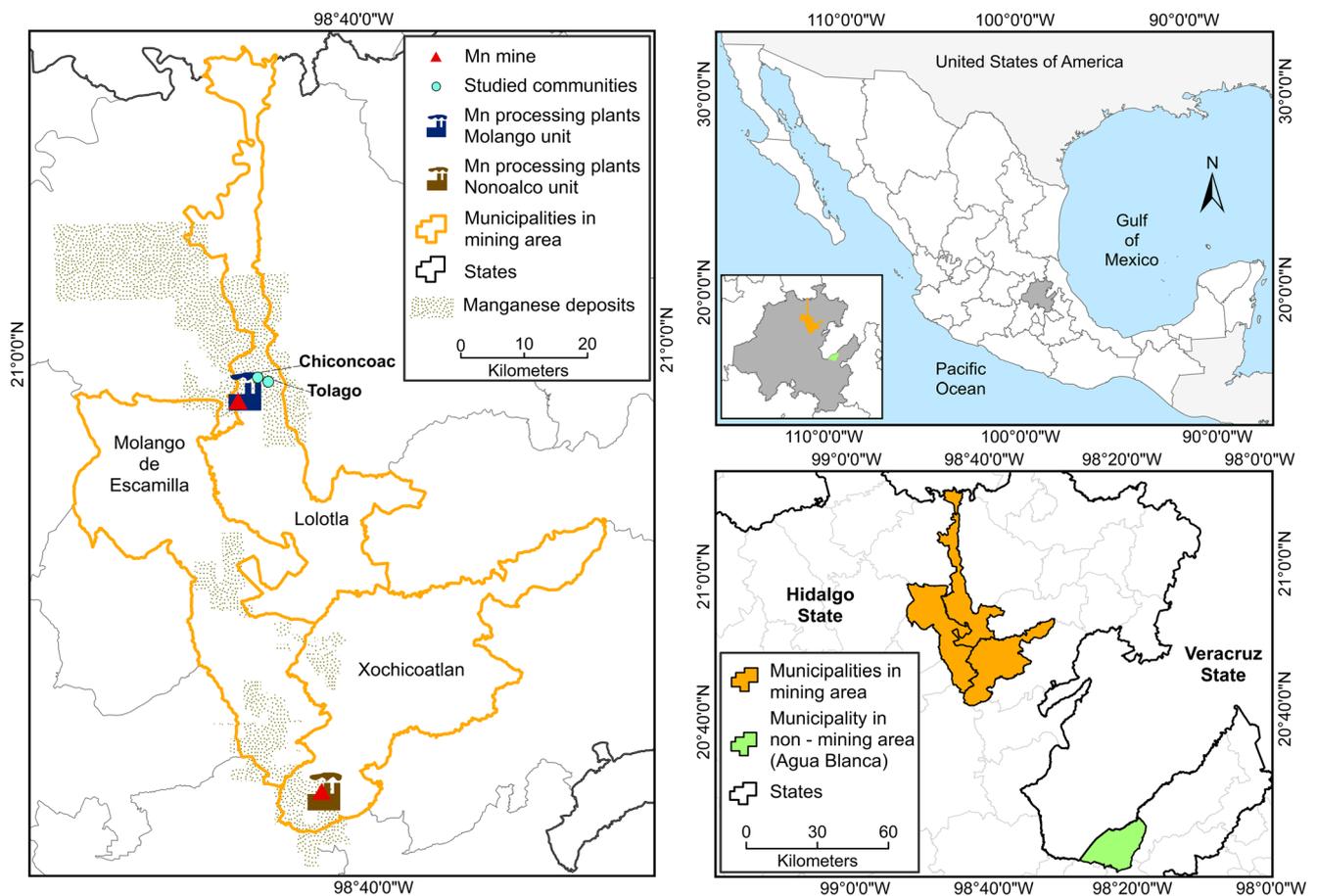


Fig. 1 Mining district of Molango, Hidalgo, Mexico

Parents of the children that participated in the study signed informed consent forms. Both studies were approved by the Bioethics and Research committees of the National Institute of Public Health, Mexico.

Socio-demographic and Child Development Variables

Socio-demographic and developmental information was collected through an interview with the child's mother (Lussier and Flessas 2001). Raven's Progressive Matrices test standardized for the Mexican population was applied to all mothers to assess intellectual function (Raven 1960).

Children's Neurocognitive Assessment. Verbal Memory and Learning

For assessment of verbal memory and learning, we used the *Children's Auditory Verbal Learning Test-2* (CAVLT-2) validated in Mexican children. This test is applied from 6 years and 6 months and up to 17 years and 11 months of age. The CAVLT-2 has been used to determine problems in skills related to verbal memory and learning in children (Talley 1997; Lezak et al. 2012).

The test took approximately 30 min, depending on the abilities of each child. It was administered and scored by a pediatric neuropsychologist. The neuropsychologist has a master's degree, was trained in pediatric neuropsychology, and has 10 years of clinical experience. At each school, testing was carried out in a classroom, with adequate lighting and minimum external noise (Lezak et al. 2012).

The CAVLT-2 has two lists of free-recall words (A and B) with 16 words each, and one recognition memory list with 32 words. The words of the free-recall lists are composed of children's common vocabulary, grouped into four semantic categories (body parts, articles of clothing, house parts, and animals). List A is presented in five trials. List B is presented as an interference test, and at the end of this, the child is asked to name the largest number of words contained in list A (immediate recall). After the immediate recall, the test is interrupted for 20 min. Once the time has elapsed, the child is asked to recall the largest number of words from list A (delayed recall); then, the child is asked to recognize the words from list A within a list of words of recognition (Talley 1997; Lezak et al. 2012).

In each of the different parts of the test, the child gets a raw score, which is transformed into the standard score according

to his/her chronological age. These scores allow calculating the learning curve (generated with the standard score of five trials), the level of learning, interference trial, immediate recall, delayed recall, immediate memory span, recognition accuracy, total intrusions, and total perseveration (Talley 1997; Lezak et al. 2012).

Exposure Assessment

To determine environmental exposure to Mn, hair and blood samples were obtained in the same session, 1 week after the cognitive assessment.

Hair Manganese

Approximately 0.5 g of hair was obtained from the occipital region as close as possible to the scalp of each child and stored in plastic bags until the analysis for determining their Mn levels. The determination was carried out as described previously by Menezes-Filho et al. (2009), and Riojas-Rodríguez et al. (2010) Samples were washed thrice by vigorous agitation with 2% Triton X-100 solution and then rinsed two times with deionized water. Samples were dried in an oven at 60 °C, cut from the nearest side to the scalp, weighed (200 mg), and then placed in a polyethylene tube (metal-traces free), adding 500 µL of concentrated nitric acid (Suprapur, Merck, Mexico). After acidic digestion of samples for 30 min at 60 °C, the resulting solution was analyzed in the graphite-furnace atomic absorption spectrophotometer analyst 600, (Perkin Elmer). Quality control for Mn determination was verified using a biological reference standard (bovine liver 1577b, National Institute of Standards and Technology, Gaithersburg, MD, USA); this biological matrix-based reference material was digested and analyzed in the same session as the samples. The quantification limit considered for hair manganese (MnH) was 0.5 µg/g.

Blood Lead

The level of lead in whole blood (PbB) was determined in the above described atomic absorption spectrophotometer system using a specific light source for Pb. Samples of blood were diluted with a matrix modifier solution consisting of 0.05% Triton X-100. Quality control was implemented by the analysis of blood with known amounts of Pb from the Wisconsin State Lab's Hygiene Program. Resulting levels are expressed as micrograms of Pb per deciliter of blood (Montes et al. 2008). Analytical determinations were considered valid only if metal measurements were between 95 and 100% of the value provided in the analysis certificate. Samples were analyzed in duplicate, with less than 10% standard deviation and the quantification limit established for PbB was 1 µg/dL.

Hemoglobin

Levels of hemoglobin (Hb) in blood samples were determined by the routine procedure used in the clinical laboratory facilities of the National Institute of Neurology and Neurosurgery, México. Hemoglobin normal reference value was 13.5 g/dL for healthy children, a cut-off for iron deficient anemia (Valenzuela et al. 2010).

Statistical Analysis

Statistical analyses included data from 265 schoolchildren with complete data (CAVLT-2 scores and biomarkers). MnH levels were transformed in tertiles (1st ≤ 0.72 µg/g; 2nd 0.73 to 3.95 µg/g; 3rd 3.96 to 48 µg/g). The sociodemographic characteristics, exposure biomarkers, and CAVLT-2 standard scores were compared between the different tertiles using the ANOVA or chi-square test, according to the type of variable. Linear regression models were conducted to explore the association of MnH and the CAVLT-2 standard scores.

The models included potential confounders selected according to prior knowledge: PbB (µg/L), HbB (g/dL), child's age (months) and sex, and the raw score of the mother's Raven test. The interaction terms between MnH exposure and PbB, children's age, gender, and phase of evaluation were tested considering a p value ≤ 0.10 . We assessed the goodness of fit and conducted residual diagnosis and influential observations for each model. Analyses were conducted using the statistical package STATA (version 14; Stata Corp, College Station, TX, USA.).

Results

Characteristics of the Study Population

Table 1 shows the sociodemographic characteristics of the study population stratified by MnH in tertiles. The mean age of the children was 9 ± 1.3 years old, 52% of the total population were girls. A significant difference was observed in the years of schooling; children from the second tertile showed more years of schooling compared with children from the other two tertiles. Hb levels were in the normal clinical range in all children, the mean level of Hb was 13.86 ± 0.82 g/dL.

The mean age of the schoolchildren's mothers was 34 years old. Differences were observed in the years of schooling of mothers; the mothers from the second tertile had more years of schooling compared with mothers from the first and third tertile. A significant difference was observed on the raw scores of the Raven test; the mothers from the first tertile had the highest raw scores.

Table 1 Sociodemographic characteristics and exposure biomarkers of the study population stratified by MnH in tertiles

Characteristics	MnH			<i>p</i> value
	1st tertile ≤ 0.72 µg/g <i>n</i> = 90	2nd tertile 0.73 to 3.95 µg/g <i>n</i> = 88	3rd tertile 3.96 to 48 µg/g <i>n</i> = 87	
Schoolchildren				
Age (years) P50(P25–P75)	9 (8–10)	9 (8–10)	9 (8–10)	0.13
Girls <i>n</i> (%)	51 (56.67)	51 (57.95)	36 (41.38)	0.05
Education (years) P50 (P25–P75)	4 (3–6)	5 (4–6)	4 (3–5)	≤ 0.01
Hb (g/dL) $\bar{x} \pm SD$	14.09 ± 0.92	13.84 ± 0.73	13.65 ± 0.75	≤ 0.01
Lolotla municipality <i>n</i> (%)	8 (6)	49 (34)	87 (60)	≤ 0.01
Agua Blanca municipality <i>n</i> (%)	82 (68)	39 (32)	0 (0)	≤ 0.01
PbB (µg/dL) $\bar{x} \pm SD$	6.61 ± 3.53	4.58 ± 3.82	3.87 ± 2.43	≤ 0.01
Mothers				
Age (years) P50 (P25–P75)	34 (30–40)	32.5 (29.5–38)	33 (29–37)	0.11
Education (years) P50 (P25–P75)	6 (4–9)	7.5 (6–9.5)	6 (3–9)	≤ 0.01
Raven raw score P50 (P25–P75)	22 (16–27)	19 (14–24)	17 (14–21)	≤ 0.01

SD, standard deviation

p value ANOVA or chi-square test

Exposure Biomarkers

Significant differences were found in MnH and PbB levels. The mean of MnH in all schoolchildren was 5.04 ± 7.47 µg/g. Sixty percent of schoolchildren from Lolotla municipality had

MnH levels greater than 3.96 µg/g. In contrast, 68 % of children from Agua Blanca municipality had levels lower than 0.72 µg/g. The mean of children's PbB levels was 5.04 ± 3.51 µg/dL; the highest levels of PbB were identified in the schoolchildren of the first tertile.

Table 2 CAVLT-2 standard and raw scores for schoolchildren stratified by MnH in tertiles

Subtests	MnH			<i>p</i> value
	1st tertile ≤ 0.72 µg/g <i>n</i> = 90 P50 (P25–P75)	2nd tertile 0.73 to 3.95 µg/g <i>n</i> = 88	3rd tertile 3.96 to 48 µg/g <i>n</i> = 87	
Learning curve				
Trial 1	93 (81–109)	100 (85–112)	89 (79–100)	≤ 0.01
Trial 2	96 (86–107)	96 (89–110)	89 (79–100)	≤ 0.01
Trial 3	99.5 (88–109)	101 (88–111)	91 (77–98)	≤ 0.01
Trial 4	103 (94–114)	103 (91–110.5)	93 (81–103)	≤ 0.01
Trial 5	103 (94–116)	103 (94–113)	96 (82–106)	≤ 0.01
Summary scales				
Level of learning	103 (92–112)	102.5 (93–112)	92 (80–100)	≤ 0.01
Interference trial	94 (83–104)	95 (83.5–107)	84 (81–93)	≤ 0.01
Immediate recall	103 (93–115)	99.5 (90–114)	93 (82–103)	≤ 0.01
Delayed recall	101.5 (92–114)	100.5 (89–110.5)	94 (82–106)	≤ 0.01
Immediate memory span	93 (82–101)	96 (85–108)	84 (76–92)	≤ 0.01
Recognition accuracy*	30 (28–31)	30 (28–31)	29 (26–30)	0.01
Total intrusions*	2 (1–6)	3 (1–6.5)	3 (0–5)	0.48
Total perseveration*	7 (4–14)	10 (5.5–16.5)	7 (3–13)	0.07

p value ANOVA

* Raw score

CAVLT-2 Scores

We observed significant differences for CAVLT-2 scores between Mn tertiles, with the lowest scores for the schoolchildren in the third tertile, except for the raw score of the total intrusions and total perseverations (Table 2).

Association between MnH and CAVLT-2

Table 3 shows the results of the linear regression models. The main exposure variable was MnH. All models were adjusted for PbB ($\mu\text{g/L}$), HbB (g/dL), child's age (months), sex, and the raw score of the mother's Raven test. MnH was associated with a significant decrease in most of the CAVLT-2 scores. MnH third tertile (3.96 to 48 $\mu\text{g/g}$) was associated with an average of 5-to 9-point decrease in the scores of the trials 1–5 of the learning curve, level of learning, interference trial, immediate recall, delayed recall, immediate memory span, and recognition accuracy. We did not find a significant association between MnH levels and raw scores of the recognition accuracy, total intrusions, and total perseveration (results not shown). An interaction between MnH and PbB, sex, and time of evaluation were tested, but no interaction was significant.

Discussion

This study confirms the negative association between Mn exposure and verbal memory and learning in schoolchildren assessed with CAVLT-2 as reported previously by Torres-Agustín et al. (2013). This study, with increased sample size and a larger variability in Mn exposure levels, shows a linear association. We observed an adverse effect on verbal memory and learning at lower Mn levels. The functional effects related to these findings suggest deficits in the short-term memory function, reduced verbal learning, deficits in cognitive flexibility and short-term memory skills, deficits to retrieve verbal information, and difficulty in the initial encoding information into long-term memory.

Studies in non-human and human models have demonstrated the role of the basal ganglia, the dorsolateral prefrontal pathway and the frontal lobe on the memory, learning, and cognitive flexibility; we know that Mn causes neurotoxicity in these structures. The dorsolateral prefrontal pathway connects the basal ganglia and frontal lobe; this pathway regulates the performance of these structures through different neurotransmitters like GABA, glutamate, and dopamine (Dobson et al. 2004; Guilarte and Chen 2007; Afifi 2006; Bowman et al. 2011; Guilarte 2013). Probably, this mechanism is affected, and it has influenced the performance of verbal memory and learning in these schoolchildren.

Table 3 Results of linear regression for CAVLT-2 scores with respect to MnH in tertiles

Subtests	MnH				PbB		R^2 adjusted
	2nd tertile		3rd tertile		β (95% CI)	<i>p</i> value	
	0.73–3.95 $\mu\text{g/g}$		3.96 to 48 $\mu\text{g/g}$				
β (95% CI)	<i>p</i> value	β (95% CI)	<i>p</i> value	β (95% CI)	<i>p</i> value		
Learning curve							
Trial 1	3.29 (–2.54–9.13)	0.27	–6.89 (–13.49–0.30)	0.04	–0.68 (–1.52–0.15)	0.11	0.05
Trial 2	2.97 (–2.02–7.95)	0.24	–6.34 (–11.97–0.70)	0.03	0.11 (–0.60–0.83)	0.76	0.05
Trial 3	2.21 (–2.90–7.31)	0.40	–8.34 (–14.11–2.57)	0.01	0.18 (–0.55–0.91)	0.63	0.06
Trial 4	0.83 (–4.28–5.93)	0.75	–8.72 (–14.49–2.95)	≤ 0.01	0.67 (–0.06–1.40)	0.07	0.10
Trial 5	0.46 (–4.32–5.23)	0.85	–9.19 (–14.59–3.80)	≤ 0.01	0.59 (–0.09–1.28)	0.09	0.10
Summary scales							
Level of learning	1.53 (–3.27–6.34)	0.53	–9.24 (–14.67–3.81)	≤ 0.01	0.57 (–0.12–1.26)	0.11	0.11
Interference trial	3.20 (–1.39–7.80)	0.17	–8.58 (–13.78–3.39)	≤ 0.01	0.08 (–0.58–0.73)	0.82	0.08
Immediate recall	–0.95 (–6.09–4.20)	0.72	–8.04 (–13.85–2.23)	0.01	0.69 (–0.04–1.43)	0.07	0.07
Delayed recall	–0.21 (–5.13–4.71)	0.93	–5.53 (–11.08–0.03)	0.05	0.93 (0.23–1.64)	0.01	0.08
Immediate memory Span	3.36 (–1.94–8.66)	0.21	–9.33 (–15.32–3.33)	≤ 0.01	–0.48 (–1.24–0.28)	0.22	0.07

Linear regression models ($n = 265$): all models were adjusted by PbB ($\mu\text{g/dL}$), HbB (g/dL), child's age (months), sex, phase, and raw score of the mother's Raven test

1st tertile ($\leq 0.72 \mu\text{g/g}$) was considered the references tertile

Riojas-Rodríguez et al. (2010) reported an association between high levels of MnH and poor intellectual performance in schoolchildren. In addition, they observed a differentiated effect of MnH between girls and boys on the verbal intelligence quotient. The effect was more significant in younger girls than boys. With our findings, we could propose that these decreased outcomes were related to the poor performance on verbal memory and learning, which affected girls' verbal development particularly.

Some studies have reported a differentiated effect in some cognitive processes by sex; however, we did not find this type of differentiation in the performance of verbal memory and learning of the children in this study (Riojas-Rodríguez et al. 2010; Oulhote et al. 2014b; Menezes-Filho et al. 2014; Hernández-Bonilla et al. 2016; Carvalho et al. 2018).

We found a negative association between MnH levels and verbal memory and learning assessed in schoolchildren, similar to that described in other papers. Wright et al. (2006) reported an inverse association between low MnH levels and verbal memory and learning in children living near a toxic waste site. Carvalho et al. (2014) and Oulhote et al. (2014a) observed a negative association between MnH levels in children environmentally exposed and their performances on task attention, short-term verbal memory, and working memory. In a linear analysis, we observed an effect on verbal memory and learning and MnH levels higher than 3.96 µg/g. Bouchard et al. (2007a) also reported a significant association with increased hyperactive and oppositional behaviors in the classroom with MnH levels higher than 3.00 µg/g.

Currently, we are aware of the neurotoxic and neurocognitive effects related to Pb exposure in children (ATSDR 2007). In our study, the schoolchildren had mean PbB levels of 5.04 ± 3.5 µg/dL. We observed that 44% of these children had levels higher than 5 µg/dL. There are two possible explanations for this finding; the first one is related to food cooking in lead-based glazes coated pottery, which is a widespread practice in Mexican rural population. The second possibility could be related to soil pollution. In Agua Blanca municipality, the predominant soils are luvisols; this kind of soil has a higher content of clays allowing greater retention of heavy metal (Zúñiga 1999; INEGI 2009; Caravanos et al. 2014). We found an unexpected result in our regression models, where a positive association between PbB and delayed recall scores (β 0.93; CI 95% 0.23–1.64) were observed. We evaluated the interaction between Mn and Pb, but it did not reach statistical significance. This finding suggests that the effects on verbal memory and learning observed herein are mainly related to Mn exposure.

Our study has some limitations. We used a cross-sectional design, which does not allow one to establish a temporal relationship between environmental exposure to Mn and the effects on verbal memory and learning. The second is due to the complexity of our study phenomenon. We consider it

important to measure other variables that may be influencing in Mn effect on children cognitive development. In addition, the possibility of selection bias was decreased since, in both studies, the population was randomly selected.

Conclusion

The results of this study suggest that Mn environmental exposure is associated with deficits in verbal memory and learning in schoolchildren exposed environmentally to Mn. Therefore, it is suggested that corrective actions should be taken to reduce the concentrations of Mn in the air in the mining district and thus lower the effect of this metal on the pediatric population.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Bioethics and Research Committees This study was approved by the Bioethics and Research Committees of the National Institute of Public Health from Mexico.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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