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Poorer cognitive function and environmental airborne Mn exposure determined by biomonitoring and personal environmental monitors in a healthy adult population

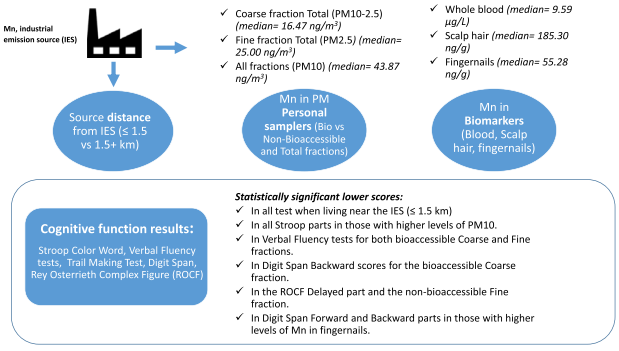
Laura Ruiz-Azcona [a], Bohdana Markiv [b], Andrea Expósito [b], Ana Pozueta [c,d], María García-Martínez [c,d], Ignacio Fernández-Olmo [b], Miguel Santibáñez [a,e,*]

[a] Global Health Research Group. Dpto Enfermería, Universidad de Cantabria, Avda. Valdecilla, s/n., 39008 Santander, Cantabria, Spain
[b] Dpto. de Ingenierías Química y Biomolecular, Universidad de Cantabria, Avda. Los Castros s/n, 39005 Santander, Cantabria, Spain
[c] Service of Neurology, IDIVAL, University Hospital Marqués de Valdecilla, University of Cantabria, 39008 Santander, Spain
[d] CIBERNED, Network Center for Biomedical Research in Neurodegenerative Diseases, 28031 Madrid, Spain
[e] Nursing Research Group, IDIVAL, Calle Cardenal Herrera Oria s/n, 39011 Santander, Cantabria, Spain

HIGHLIGHTS

- Volunteers living near a ferromanganese alloy plant wear personal samplers for 24 h.
- Fine (PM2.5) and coarse (PM10–2.5) and bioaccessible fractions were differenciated.
- Blood, hair and fingernails samples were also obtained.
- A battery of five cognitive tests was applied.
- Our results support poorer cognitive function among those with higher Mn exposures.

GRAPHICAL ABSTRACT



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ABSTRACT

Background/aim: In the Santander Bay (Cantabria, northern Spain), a ferromanganese alloy plant is located. Our objective was to characterize the Mn personal exposure of adult healthy volunteers living in this highly Mn exposed region, and to determine its association with a poorer cognitive function.
Methods: Cross-sectional study analyzing 130 consecutive participants. Cognitive function was assessed by Stroop Color Word, Verbal Fluency tests, Trail Making Test (TMT), Digit Span (WAIS III) and Rey Osterrieth Complex Figure (ROCF) tests and crude scores were standardized according to NEURONORMA norms. Exposure to Mn was assessed in terms of source distance, by Personal Environmental Monitors (PEMs) allowing the separation of fine (PM2.5) and coarse (PM10–2.5) particles (obtaining the bioaccessible fraction by *in-vitro* bioaccessibility tests), and by biomarkers (blood, hair and fingernails). Age, sex, study level and number of years of residence were predefined as confounding variables and adjusted Mean Differences (MDs) were obtained.
Results: Statistically significant lower scores (negative MDs) in all test were observed when living near the industrial emission source, after adjusting for the predefined variables. Regarding PEMs results, statistically significant lower scores in all Stroop parts were obtained in participants with higher levels of Total Mn in All fractions (PM10). For Verbal Fluency tests, negative MDs were obtained for both bioaccessible fractions. Digit Span Backward scores were lower for those with higher levels in the bioaccessible coarse fraction, and negative MDs were also observed for the ROCF Delayed part and the non-bioaccessible fine fraction. As regards to Mn in fingernails, adjusted MDs of −1.60; 95%

* Corresponding author at: Global Health Research Group. Dpto Enfermería, Faculty of Nursing, Universidad de Cantabria-IDIVAL, Avda. Valdecilla, s/n., 39008 Santander, Cantabria, Spain.
E-mail address: miguel.santibanez@unican.es (M. Santibáñez).

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CI (−2.57 to −0.64) and −1.45; 95%CI (−2.29 to −0.61) for Digit Span Forward and Backward parts were observed.
Conclusions: Our results support an association between poorer cognitive function and environmental airborne Mn exposure.

1. Introduction

Neurological and neuropsychological negative health effects have been described in relation to high levels of occupational Manganese (Mn) (Bowler et al., 2006a, 2006b; Dlamini et al., 2020; Lucchini et al., 1999; Mergler et al., 1994; Roels et al., 1987; Williams et al., 2012), even though it is an essential trace element found in the human body (Chen et al., 2018). In addition to evidence from occupational studies, a few epidemiological studies have also been published in recent decades with regard to environmental airborne exposure (Fernández-Olmo et al., 2021). Neurological and neuropsychological outcomes can be classified in these studies into cognitive, motor, olfactory and emotional function, and mood (Fernández-Olmo et al., 2021). Recently, six studies have been meta-analyzed in a correlation or standardized mean difference (MD) approach (Bowler et al., 2012; Bowler et al., 2015; Ghazali et al., 2013; Iqbal et al., 2018; Lucchini et al., 2014; Viana et al., 2014) regarding cognitive function and environmental airborne Mn exposure (Ruiz-Azcona et al., 2021a), with study populations from United States (US) (Bowler et al., 2012; Bowler et al., 2015); Malaysia (Ghazali et al., 2013); Pakistan (Iqbal et al., 2018), Italy (Lucchini et al., 2014) and Brazil (Viana et al., 2014); living in low population density areas in the vicinity of industrial emission sources (IES) of Mn such as Mn alloys smelters (Bowler et al., 2012; Bowler et al., 2015; Lucchini et al., 2014; Viana et al., 2014), or Mn ore processing plants (Bowler et al., 2012, 2015).

Most of these studies were carried out near industrial sources of airborne Mn. Although other routes of environmental exposure to Mn may occur, such as ingestion of food and/or water with relatively high Mn content (Bouchard et al., 2011; Ntihabose et al., 2018), the inhalation route of exposure is the most important one in the vicinity of industrial sources of particulate matter (PM)-bound Mn (Fernández-Olmo et al., 2021). Stationary PM samplers have been classically used to determine metal(loid)s inhalation exposure, but they are only a surrogate of personal air sampling (Fulk et al., 2016). Personal Environmental Monitors (PEMs) also known as PM personal samplers, solve this limitation (Graney et al., 2004; Haynes et al., 2012; Lucchini et al., 2012; Pollitt et al., 2016; Solís-Vivanco et al., 2009), but to the best of our knowledge there are only two specific studies on the relationship between environmental airborne Mn personal exposure and cognitive function. Lucchini et al. (2014) performed 24 h personal air sampling of PM10, finding negative MDs in some cognitive tests such as Trail Making Test (TMT). Solís-Vivanco et al. (2009) placed PEMs in some participants' homes to measure the Mn levels, finding a statistically significant association for attention impairment (Digit Span test) in its Odds Ratio (OR) approach. On the other hand, the total content of metals is determined in these studies, instead of measuring their bioaccessible concentration. As this bioaccessible fraction potentially represents better the exposure risk, it would be interesting to add in exploratory epidemiologic studies (Expósito et al., 2021a; Hernández-Pellón et al., 2018; Weggeberg et al., 2019). In addition to PEMs, the personal characterization of exposure can also be performed through biomarkers. Blood (Bowler et al., 2012; Lucchini et al., 2014), scalp and axillary hair (Viana et al., 2014), urine (Lucchini et al., 2014) and fingernails and saliva (Viana et al., 2014) have been used in the published studies with different results (Bauer et al., 2020a; Fernández-Olmo et al., 2021; Haynes et al., 2015; Ntihabose et al., 2018; Viana et al., 2014).

The presence of a ferromanganese alloy smelter in the Santander bay (northern Spain), where the capital of the region, Santander (172,000 inhabitants) is located, has led to historical elevated levels of airborne Mn, sometimes higher than the annual reference guideline given by WHO (150 ng/m^3). For example, in Maliaño (9535 inhabitants), the town where the plant is located, an annual mean of 232 ng/m^3 was reported in 2015 (Hernández-Pellón and Fernández-Olmo, 2019a); although in Santander the levels are lower, the mean measured in 2015 (60.8 ng/m^3) exceeded the Reference Concentration (RfC) given by US EPA (*i.e.*, 50 ng/m^3). These elevated levels of airborne Mn have also been associated with relatively high concentrations of PM_{10}, mainly in the town of Maliaño, where exceedances of the daily limit value of 50 μg/m^3 set by the EU regulation (Directive 2008/50/EU) were common before a local air quality plan was approved in 2012 (Fernández-Olmo et al., 2016). Due to the high emission of airborne Mn in the Maliaño area, high atmospheric deposition rates of Mn (2606 μg/m^2/day) and high Mn levels in local soil (4333 mg/kg) have also been reported (Fernández-Olmo et al., 2015). Water ingestion is not considered a source of Mn, as its concentration in local tap water is low (0.38 μg/L) as measured in 2020, far below the concentrations reported in other studies, in a range of 1–2700 μg/L (Bouchard et al., 2011; Ntihabose et al., 2018).

Therefore, the objective of this study was to analyze the impact of environmental Mn exposure on cognitive function in adults living in a region characterized by high levels of airborne Mn by using PEMs and differentiating between bioaccessible and non-bioaccessible fractions and between fine and coarse modes, in addition to biomarkers of exposure (whole blood, scalp hair and fingernails).

2. Methods

2.1. Design, area of study and participants

A cross-sectional design was used. The area of study (Santander Bay, Cantabria, northern Spain) has been described elsewhere (Arruti et al., 2010, 2011a, 2011b; Hernández-Pellón and Fernández-Olmo, 2019a, 2019b). Those living in the town of Maliaño within a radio of approximately 1.5 km from the IES (a ferromanganese alloy plant that produces more than 100 kt of ferromanganese and silicomanganese, annually) were considered as the highly exposed group; whereas those living in the city of Santander located between 5 and 10 km from the same IES, were considered as moderately-exposed. A map of the study area is depicted in Fig. 1, where the location of volunteers' residence and the IES of Mn is shown.

Likewise, the recruitment and selection of participants has been described (Expósito et al., 2021b; Ruiz-Azcona et al., 2021b). Fig. S1 shows the flow chart to obtain the final study population (n = 130) in compliance with the inclusion criteria of age (between 18 and 75 years old); and the fulfilment of at least one of the following exposure criteria:

- Residence criterion 1: At least one year of current residence in the same place, located up to 10 km away from the IES (n = 123, 94.6%).
- Residence criterion 2: ≥10 years of residence during the last 15 years located up to 10 km away from the IES (n = 118, 90.8%).
- Workplace criterion (minimum daily working time of 8 h): ≥10 years of workplace during the last 15 years located up to 10 km away from the main emission source (n = 128, 98.5%).

The following exclusion criteria were considered:

- Neurodegenerative disease diagnosed (cognitive impairment, multiple sclerosis, Alzheimer's dementia, Huntington's chorea and Parkinson's disease) or psychiatric condition (schizophrenia, major psychiatric diagnosis, eating disorder and bipolar disorder).
- Medical sick leave that interferes with cognitive or motor function or makes displacement not possible.

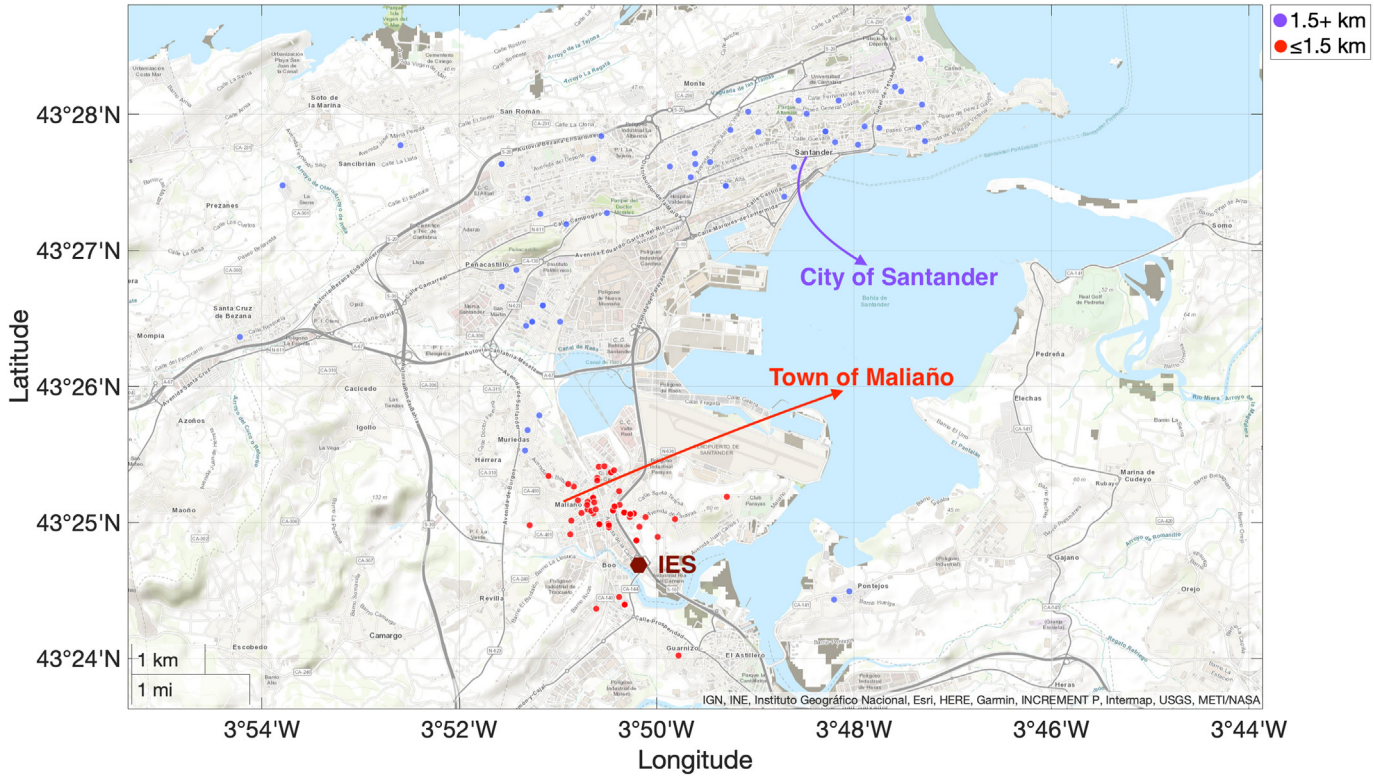

Fig. 1. Map of the study area, showing the location of volunteers' residence and the industrial emission source (IES) of Mn.

- Current medication prescribed interfering with cognitive or motor function (anticonvulsive, antihistamines, neurological or psychiatric drugs).
- History of previous occupational exposure to manganese requiring medical treatment.
- Having worked in Mn related industries.
- Alcohol consumption at the moment of testing and/or other psychotropic drugs.
- Lack of understanding of the Spanish language for the purpose of completing the tests.

An informed written consent was obtained from each subject. The study was approved by the ethical committee of clinical research in Cantabria (CEIC) and by the ethical committee of research of the University of Cantabria (CEUC).

2.2. Cognitive function tests and data collection

The standardized cognitive function test battery included five tests assessing specifically attention, executive function, memory and verbal fluency (Strauss et al., 2006):

Stroop Color Word test is considered a measure of executive function and cognitive flexibility. This test consists of three parts. First part (Stroop Word) is composed of the words "RED", "GREEN", "BLUE", written randomly in black color in columns. The task is to read the words in the text as quickly as possible in the columns within 45 s. The more words correctly read, the better function. In the second part (Stroop Color) the words have been replaced by the term "XXXX" printed randomly in red, green and blue colors in the column. The task is to correctly name the color in which the "XXXX" is printed. The more colors correctly said within 45 s, the better function. In the third part (Color&Word) the words "RED", "GREEN", "BLUE" are presented but printed in red, green, and blue colors in instead of black. The color and the word do not match (*i.e.* the word "GREEN" is printed in red and blue colors, but not in green). As in the second part, the participant has to correctly identify the printed color with independence of the written word (*i.e.* if the word "GREEN" is printed in blue, the participant must say blue). The more colors correctly named, the better cognitive function (Golden, 2001).

In the Verbal Fluency tests, the total number of words that a subject is able to say during 60 s, starting with a specific letter that the evaluator decides, are computed, evaluating verbal association fluency. The letters used in this study, as usual, are P, M and R (Ruff et al., 1997). The more words, the better cognitive function.

TMT is a measure of attention, speed and mental flexibility. The test has two parts. In part A, the participant has to draw a line connecting sequentially the numbers 1–25 as quickly as possible. In TMT-B the task is more complicated, since the participant has to draw a line connecting sequentially numbers and letters (*i.e.* 1-A-2-B-3…). Part A requires recognition of numbers, knowledge and reproduction of numerical sequences, visual processing, and motor speed; part B requires also cognitive flexibility with shifting ability. The more seconds required to complete the task the worse cognitive function (Partington and Leiter, 1949; Reitan and Wolfson, 1993).

The Digit Span, from the third version of the Wechsler Adult Intelligence Scale (WAIS III) is commonly used to measure the span of immediate recall, and it is considered a measure of attention and working memory. In the forward part, the participant must repeat in the same order, the sequences of numbers read by the evaluator previously. These sequences are up to 9 digit longer (*e.g.* 2-7-5-8-6-2-5-8-4). In the Backward part, the sequences must be repeated by the participant in reverse order (Wechsler, 2001). The longer the sequence repeated correctly in each part, the better the scores and the cognitive function.

Finally, the purpose of Rey Osterrieth Complex Figure (ROCF) is to assess visual-spatial constructional ability and visual memory. In the Copy part of this test, the participant must copy the figure itself. In the Delayed part, the participant must repeat the figure after 30 min, this time using his/her memory (without possibility of seeing the figure again) (Rey, 2003). The more parts copied and repeated, the better the scores and the cognitive function.

With regard to data collection, an individual first appointment was scheduled with each of the 130 volunteers at a Testing room of the

University of Cantabria (UC) that met the privacy needs for cognitive evaluation. During each of the appointments, the sequence of steps was as follows: signature of the informed written consent form. Personal interview using a structured questionnaire with socio-demographic data, place of residence and work and years residing, work history, medical history, pharmacological treatments, toxic (tobacco, alcohol and other drugs use) and dietary habits. Cognitive tests battery (always in the same order). Tests were similarly measured, administered and evaluated among all participants, following standardized instructions by a single investigator (L.R.-A), with previous experience in carrying out neuropsychological tests in epidemiological studies (Herrero-Montes et al., 2019) and trained by neurologist and neuropsychologist of the neurology service from the Hospital Universitario Marques de Valdecilla (HUMV). Raw test scores were converted into age and years of education adjusted scaled scores (NSSA) to ensure a normal distribution, with NEURONORMA norms, allowing for demographic adjustments for ages of 18–90 years (for each age range a cumulative frequency distribution of the raw scores was generated), of neurologically normal adults, native Spanish language speakers, and educated in Spain. Raw scores were assigned percentile ranks in function of their place within the distribution. Subsequently, percentile ranks were converted to scaled scores (from 2 to 18) based on percentile ranges. A scale score higher than 6 indicates normality (Peña-Casanova et al., 2009). At the end of the tests, a PEM was handed out and fitted, which the subject was required to carry with him/her for at least 24 h. The next day, the participant handed over the PEM and then biological samples were collected. Specifically, a blood sample, a hair sample from the occipital part of the scalp and fingernails were taken.

2.3. Biomarkers sampling and analysis

Biological samples were taken in a laboratory of the Faculty of Nursing of the University of Cantabria, the day after the neuropsychological testing and after the delivery of the personal sampler of particulate matter that each subject had to carry with them for at least 24 h. Lithium heparin monovettes (Sarstedt, Nümbrecht, Germany) were used to collect whole blood samples (7.5 mL) that were stored at 4 °C until analysis for a maximum of 14 days. Then, samples were analyzed by ICP/MS after dilution with an alkaline solution ((2% (w/v) 1-butanol, 0.05% (w/v) EDTA, 0.05% (w/v) triton X-100 and 1% (w/v) NH_4OH)) as described in González-Antuña et al. (2017). A blood/alkaline solution ratio of 1/10 (w/w) was used expect for Fe, Zn and Cu.

Hair samples (approximately 0.5 g) were obtained from the occipital part of the scalp of each subject using ceramic scissors (Kyocera advanced ceramics CS-124) and stored in sterile propylene bottles for transport and storage. For subsequent analysis, 0.2 g were cut and analyzed to use only the hair closest to the scalp. Fingernails of both hands were cut by nail clippers after asking the volunteers to wash them with a liquid soap. Collected scalp hair and fingernails samples were exhaustively washed, according to the procedure reported by Eastman et al. (2013), removing all exogenous metals before digestion. The washing protocol consisted of five stages: sonication for 20 min with a 0.5% (*w/v*) solution of triton X-100, five times washing with ultrapure water, sonication with a 1 N HNO_3 solution, washing with the 1 N HNO_3 solution and last washing (five times) with ultrapure water. Then, samples were dried in an oven at 65 °C and digested by a microwave digestor (Milestone, Ethos One) at 200 °C using a mixture of HNO_3/H_2O_2 at a ratio 4 /1 (v/v); the extracts were analyzed by ICP/MS. Additional information on the ICP-MS determination of Mn levels in blood, hair and fingernails can be found in Markiv et al. (2020).

Analytical grade reagents provided by Merck and PanReac AppliChem (Darmstadt, Germany) were used in all the experimental procedures.

2.4. Statistical analysis

Continuous variables were described as mean and Standard Deviation (SD) and/or median and interquartile ranges (IQR). Statistical differences between groups were compared by using the Student's *t*-test (for equal or different variances, depending on the previous result in the Levene test) in the case of Mean comparisons and Medians were compared by using the U Mann Whitney Test. Categorical and discrete variables were expressed as percentages, and comparisons were performed with the Chi-square test, using Yates´ correction or Fisher's exact test, when appropriate.

The Spearman's rank correlation coefficient (rho) was used to estimate correlation between Mn exposure and cognitive test scores as continuous variables. Exposure to Mn was dichotomous categorized according to the median (lower *versus* higher values) and crude and adjusted MDs with their 95% confidence intervals (CI); 95% CIs were calculated by using linear regression models in which the quantitative results in each cognitive test were treated as dependent variables in each model, and Mn exposure was introduced as a binary variable (0 = lower values; 1 = higher values). Age (as a continuous variable), sex, and study level (ordinal categorized) were established as confounders and introduced as covariates in the models to obtain adjusted MDs. In addition, to estimate the strength of associations, cognitive tests scores were dichotomous divided into lower & higher values according to medians and adjusted ORs with their 95%CI were calculated by using unconditional logistic regression models in which the binary results in each cognitive test (lower & higher test scores) were treated as dependent variables in each model, being Mn exposure also introduced as a binary variable. Age, sex, and study level were then introduced as covariates in the models to obtain the adjusted ORs. A flow chart for the statistical analysis is presented as Fig. S2.

All tests were two-tailed, setting the level of statistical significance at 0.05. Analysis were performed by using SPSS statistical software package 24.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Basal characteristics of study population

The overall mean age of the study participants was 41.72 years (SD 13.97) with an overall mean of 15.26 years residing in the same last address (SD 13.71). Ninety five out of 130 participants (73.1%) were female. Regarding study level: 55.4% had university studies (14.6% Bachelor's Degree and 40.8% University Degree), 23.1% high school education, 16.9% secondary school education and 4.6% primary education. In terms of employment status, most of participants (74.6%) were full-time employed (see Table 1).

3.2. Cognitive results

For Stroop Color Word test, overall arithmetic mean scores standardized by NEURONORMA were 10.51 (SD = 2.67), 10.38 (SD = 2.59) and 10.69 (SD = 2.77) for each part (Word, Color, Color&Word), with medians of 11.00, 11.00 and 10.00 respectively. In relation to the Verbal Fluency tests, overall NEURONORMA mean scores were 10.55 (SD = 2.56), 10.37 (SD = 2.27) and 10.48 (SD = 2.45) in each test (P, M, R), with medians of 10.00, 11.00 and 11.00. TMT mean score was 12.47 (SD = 2.85) for the part A and 11.88 (SD = 2.32) for part B, with medians of 12.5 and 12.00. Digit Span forward mean scores were 9.77 (SD = 2.57) with a mean of 9.88 (SD = 2.26) for the Backward part, with medians of 10.00 and 10.00 respectively. ROCF copy and delayed overall mean scores were 15.89 (SD = 3.09) and 9.48 (SD = 2.03), with medians of 17.00 and 9.50 (see Table S1).

When comparing basal characteristics of study population as a function of cognitive test results, statistically significant differences were observed between ROCF test scores and age. For Stroop Color Word, Verbal Fluency and ROCF test results, statistically significant differences in relation to study level were also observed, even though NEURONORMA includes both age and study level in its standardization. In addition, the longer the number of years of residence, the worse the Stroop Color Word test results. No significant differences were observed in relation to the other baseline characteristics (see Tables S2 to S8).

Table 1
Characteristics of the study population as a function of sex.

	Female		Male		Total		p value
	n = 95		n = 35		n = 130		
Age (Mean, SD)	40.29	13.7	45.57	14.15	41.72	13.97	0.056
(Range: min, max)	20	71	21	71	20	71	
Study level (n, %)							0.068
Primary education	5	5.3%	1	2.9%	6	4.6%	
Secondary education	12	12.6%	10	28.6%	22	16.9%	
High school level	22	23.2%	8	22.9%	30	23.1%	
University studies (Bachelor's Degree)	18	18.9%	1	2.9%	19	14.6%	
University studies (University Degree)	38	40.0%	15	42.9%	53	40.8%	
Years residing (Mean, SD)	16.08	14.77	12.91	9.86	15.26	13.71	0.242
(range: min, max)	1	71	2	38	1	71	
Employment status							0.072
Employed full-time	72	75.8%	25	71.4%	97	74.6%	
Unemployed	2	2.10%	3	8.6%	5	3.8%	
Housewife	5	5.3%	0	0.0%	5	3.8%	
Retired	7	7.4%	6	17.1%	13	10.0%	
Full-time student	9	9.5%	1	2.9%	10	7.7%	
Smoking status							0.973
Non-smoker	61	64.2%	23	65.7%	84	64.6%	
Former	16	16.8%	6	17.1%	22	16.9%	
Current	18	18.9%	6	17.1%	24	18.5%	
Alcohol status							0.005
Never	59	62.1%	12	34.3%	71	54.6%	
Ever	36	37.9%	23	65.7%	59	45.4%	
Average of pure ethanol (g/week)							0.006
0 g/week	59	62.0%	12	34.3%	71	54.6%	
1–24 g/week	16	16.8%	6	17.1%	22	16.9%	
25–74 g/week	19	20.0%	14	40.0%	33	25.4%	
≥75 g/week	1	1.1%	3	8.6%	4	3.1%	

3.3. Mn levels

Table S9 shows the Mn levels of participants in PEMs and biomarkers as a function of proximity to IES. All Mn determinations in PEMs (in both bioaccessible and non-bioaccessible coarse&fine and total fractions) were statistically significantly higher in participants residing at a shorter distance (≤1.5 km) from the IES compared to those residing at greater distance (>1.5 km and ≤10 km) considered as highly and moderately exposed respectively.

3.4. Associations between Mn levels and cognitive results

3.4.1. Mn levels and Stroop Color Word test results

Statistically significant lower scores in all Stroop parts (indicating worse executive function) were observed when living near the IES, after adjusting for the predefined variables with adjusted MDs of –1.63; 95%CI (−2.54 to −0.72); −1.70; 95%CI (−2.59 to −0.81) and −1.67; 95%CI (−2.64 to −0.71) in each part of the test. In the form of ORs, the results were similarly statistically significant with 3.53; 2.88 and 3.20-fold increase of having lower scores (below the median) in the Word, Color and Color&Word parts respectively. Regarding PEMs results, statistically significant lower scores in all Stroop parts were also obtained in participants with higher levels of Total Mn in All fractions (PM10) with adjusted MDs of −1.07; 95%CI (−1.99 to −0.15); −1.29; 95%CI (−2.20 to −0.39) and −1.34; 95%CI (−2.30 to −0.37) (see Table 2). Statistically significant crude and adjusted OR were obtained for bioaccessible, Total (Bio + Non-Bio) and All fractions (PM10) on the risk of having lower scores in the Word and Color&Word parts (see Tables 2 and S11 to S13).

Regarding biomarkers, the results most favorable to our hypothesis were in fingernails, with statistical significance crude MDs in the Color and Color&Word parts. After adjusting for the age, sex and study level the adjusted MDs remained in favor of our hypothesis but diminished, losing statistical significance: adjusted MD for Color part = −0.90; 95%CI (−1.88 to 0.08), p = 0.071; adjusted MD for Color&Word part = −0.94; 95%CI (−1.99 to 0.12), p = 0.081. For scalp hair Mn nonsignificant negative crude and adjusted MDs were obtained, whereas for Blood Mn levels higher cognitive punctuations were observed (contrary to our hypothesis). In terms of OR, results were in accordance, with the higher OR being maintained for fingernails (see Tables 2 and S11 to S13).

In the correlation approach, statistically significant correlations in favor of our hypothesis were obtained (positive Spearman coefficients for source distance, and negative for the rest), except for blood Mn levels. Spearman coefficients were slightly higher in the bioaccessible fractions (see Table S14).

3.4.2. Mn levels and verbal fluency tests battery results

Statistically significant lower scores in the three Verbal Fluency tests were obtained in relation to source distance, with adjusted MDs of −1.51; 95%CI (−2.40 to −0.62; −1.47; 95%CI (−2.26 to −0.67) and −1.60; 95%CI (−2.45 to −0.74) for the Letter P, Letter M and Letter R tests respectively. Regarding PEMs results, crude and adjusted negative MDs were obtained, most of them yielding statistical significance, and being of greater magnitude for the bioaccessible fractions (see Tables 3 and S16 to S18).

Regarding biomarkers, nonsignificant negative crude and adjusted MDs were obtained for scalp hair and fingernails Mn levels, whereas for Blood Mn levels higher cognitive punctuations were observed again (contrary to our hypothesis). OR results were in accordance (>1 for scalp hair and fingernails Mn levels and <1 or close to 1 for Blood Mn levels) (see Tables 3 and S16 to S18).

A positive statistically significant correlation was obtained for Letter R in relation to source distance, and negative statistically significant coefficients were obtained for all PEMs results. For Letter P and Letter M tests, significant correlations were also observed for source distance and PEMs results with the exception of the non-bioaccessible coarse and fine fractions. Biomarkers showed negative nonsignificant correlations, with the exception of blood (see Table S14).

3.4.3. Mn levels and TMT results

Statistically significant lower scores in TMT for those living near the IES were obtained with and adjusted MD of −1.32; 95%CI (−2.34 to −0.31) for TMT A and −0.97; 95%CI (−1.78 to −0.15) for TMT B. In terms of ORs higher associations were obtained for TMT B: adjusted OR = 2.34; 95%CI (1.08 to 5.08). In relation to PEMs results, nonstatistically significant negative crude and adjusted MDs were obtained. In the form of ORs, positive associations (OR > 1) were also obtained for TMT B, whereas mixed results were observed for TMT-A (see Tables 4 and S20 to S21).

Regarding Biomarkers, scalp hair and fingernails showed negative MDs, yielding statistical significance in the case of the crude MD for fingernails in TMT-B: crude MD = −1.00; 95%CI (−1.83 to −0,17), p = 0.018. MDs for blood Mn levels were positive again. ORs were in accordance, with higher associations for TMT-B and fingernails Mn levels: Crude OR = 2.62; 95%CI (1.21 to 5.68) (see Tables 4 and S20 to S21).

Statistically significant positive correlations between TMT (A and B parts) and source distance were obtained in concordance with the MD approach. Regarding PEMs, most of the Spearman coefficients were negative in favor of our hypothesis, but without reaching statistical significance. For biomarkers, coefficients of smaller magnitude and non-statistical significance were found, expect for fingernails in part B (p = 0.028) (see Table S14).

3.4.4. Mn levels and digit span test results

Statistically significant MDs were obtained in relation to source distance for Digit Span, in both Forward: adjusted MD = −0.97; 95%CI (−1.90 to −0.04) and Backward parts: adjusted MD = −1.29; 95%CI (−2.08 to −0.49). A statistically significant OR was also obtained for Backward part, adjusted OR = 3.30; 95%CI (1.52 to 7.14). In relation to PEMs results, statistically significant MDs for Digit Span Backward and the bioaccessible coarse fraction were obtained, adjusted MD = −1.02; 95%CI (−1.81 to −0.22). In the form of OR, the association was of borderline significance:

Table 2
Mean differences for Stroop Color Word test according to Mn exposure indices and biomarkers.

	Stroop Color Word test				MDa	95%	CI	p value
	MD crude	95%	CI	p value				
Exposure indices								
Source distance from IES (≤ 1.5 *vs* 1.5+ km)								
Stroop Word	−1.66	−2.54	−0.78	<0.001	−1.63	−2.54	−0.72	0.001
Stroop Color	−1.73	−2.58	−0.87	<0.001	−1.70	−2.59	−0.81	<0.001
Stroop Color Word	−1.67	−2.59	−0.75	<0.001	−1.67	−2.64	−0.71	0.001
PM personal samplers (ng/m^3)								
Coarse fraction (PM10–2.5)								
Bioaccessible (13.62+ vs ≤ 13.61 ng/m^3)								
Stroop Word	−1.05	−1.96	−0.14	0.025	−0.87	−1.81	0.06	0.066
Stroop Color	−1.14	−2.02	−0.25	0.012	−1.06	−1.97	−0.16	0.022
Stroop Color Word	−1.02	−1.97	−0.065	0.036	−1.00	−1.97	−0.02	0.046
Non-bioaccessible (3.39+ vs ≤ 3.38 ng/m^3)								
Stroop Word	−1.14	−2.05	−0.23	0.014	−1.03	−1.93	−0.12	0.027
Stroop Color	−0.46	−0.46	0.45	0.320	−0.36	−1.26	0.55	0.438
Stroop Color Word	−0.37	−1.33	0.60	0.454	−0.30	−1.27	0.68	0.548
Total (Bio + Non-Bio) (16.48+ vs ≤ 16.47 ng/m^3)								
Stroop Word	−1.26	−2.16	−0.36	0.006	−1.10	−2.02	−0.18	0.020
Stroop Color	−1.23	−2.11	−0.35	0.007	−1.13	−2.03	−0.23	0.015
Stroop Color Word	−1.05	−2.00	−0.10	0.031	−1.00	−1.97	−0.02	0.046
Fine fraction (PM2.5)								
Bioaccessible (17.06+ vs ≤ 17.05 ng/m^3)								
Stroop Word	−0.83	−1.75	0.09	0.075	−0.68	−1.60	0.24	0.145
vStroop Color	−0.75	−1.65	0.14	0.099	−0.72	−1.63	0.19	0.118
Stroop Color Word	−1.18	−2.13	−0.24	0.015	−1.23	−2.19	−0.27	0.012
Non-bioaccessible (5.81+ vs ≤ 5.80 ng/m^3)								
Stroop Word	−0.15	−1.08	0.77	0.744	0.07	−0.86	1.00	0.879
Stroop Color	−1.13	−2.01	−0.24	0.013	−1.04	−1.94	−0.14	0.024
vStroop Color Word	−0.56	−1.53	0.40	0.250	−0.52	−1.50	0.46	0.295
Total (Bio + Non-Bio) (25.01+ vs ≤ 25.00 ng/m^3)								
Stroop Word	−1.05	−1.96	−0.14	0.025	−0.84	−1.77	0.09	0.075
Stroop Color	−0.82	−1.71	0.08	0.074	−0.72	−1.64	0.20	0.124
Stroop Color Word	−1.06	−2.01	−0.11	0.029	−1.07	−2.05	−0.09	0.033
All fractions (PM10) (43.88+ vs ≤ 43.87 ng/m^3)								
Stroop Word	−1.26	−2.16	−0.36	0.006	−1.07	−1.99	−0.15	0.023
Stroop Color	−1.41	−2.28	−0.53	0.002	−1.29	−2.20	−0.39	0.005
Stroop Color Word	−1.37	−2.31	−0.43	0.005	−1.34	−2.30	−0.37	0.007
Blood Mn (9.59+ vs ≤ 9.58 μg/L)								
Stroop Word	0.71	−0.21	1.63	0.131	0.71	−0.22	1.64	0.135
Stroop Color	1.03	0.14	1.92	0.023	0.84	−0.07	1.75	0.070
Stroop Color Word	1.15	0.20	2.10	0.018	0.96	−0.01	1.94	0.053
Scalp hair Mn (185.31+ vs ≤ 185.30 ng/g)								
Stroop Word	−0.67	−1.60	0.25	0.153	−0.55	−1.49	0.38	0.246
Stroop Color	−0.59	−1.51	0.33	0.208	−0.49	−1.44	0.45	0.304
Stroop Color Word	−0.46	−1.44	0.52	0.355	−0.35	−1.36	0.65	0.487
Fingernails Mn (555.28+ vs ≤ 555.28 ng/g)								
Stroop Word	−0.88	−1.86	0.10	0.079	−0.95	−1.97	0.07	0.066
Stroop Color	−1.03	−1.97	−0.10	0.030	−0.90	−1.88	0.08	0.071
Stroop Color Word	−1.07	−2.07	−0.07	0.037	−0.94	−1.99	0.12	0.081

IES = Industrial Emission Source. MD = Mean Difference. MDa = adjusted for age, sex and study level. *A negative MD indicates worse cognitive function in exposed to higher Mn levels.

i.e. adjusted ORa = 2.07; 95%CI (0.99 to 4.33), p = 0.053. For the rest of PEMs determinations, nonstatistically significant negative crude and adjusted MDs were obtained (see Tables 5 and S23 to S24).

Regarding Biomarkers, a negative MD was found between scalp hair Mn levels and the Digit Span Forward part: adjusted MD = −1.14; 95%CI (−2.06 to −0.21). For fingernail Mn levels, MDs were of greater magnitude and statistically significant for both Forward and Backward parts, with adjusted MDs of −1.60; 95%CI (−2.57 to −0.64) and −1.45; 95% CI (−2.29 to −0.61) respectively. Mixed results were found for blood Mn levels with negative and positive crude and adjusted MDs for the Forward and Backward parts respectively. ORs were in concordance, with higher associations for fingernails Mn levels, especially in relation to the Backward part: adjusted OR = 3.50; 95%CI (1.53 to 8.02) (see Tables 5 and S23 to S24).

In terms of correlations, a statistically significant positive correlation between the Digit Span Backward part and source distance was obtained. Regarding PEMs Spearman coefficients, all of them with the exception of the non-bioaccessible fine fraction were negative, but without reaching statistical significance. For biomarkers, statistically significant negative coefficients were found for scalp hair and fingernails Mn levels (see Table S25).

3.4.5. Mn levels and ROCF test results

A statistically significant MD was obtained in relation to source distance for the ROCF Copy part: adjusted MD = −1.49; 95%CI (−2.56 to −0.42), with OR in accordance: adjusted OR = 2.20; 95%CI (0.91 to 5.30), p = 0.078. Regarding PEMs results, negative MDs were obtained, with statistically significant results centered on the non-bioaccessible fine fraction in relation to the Delayed part: adjusted MD = −1.21; 95%CI (−1.91 to −0.51), p = 0.001 and results of borderline significance for the bioaccessible fine fraction: adjusted MD = −0.68; 95%CI (−1.39 to 0.04), p = 0.063. ORs for fine fraction results were in accordance, with a statistically significant OR for the non-bioaccessible fine fraction in relation to the Delayed part: adjusted OR = 2.63; 95%CI (1.29 to 5.37) and positive but not significant for the bioaccessible fine fraction: adjusted OR = 1.52; 95%CI (0.75 to 3.09) (see Tables S27 to S29).

Table 3
Mean differences for verbal fluency tests according to Mn exposure indices and biomarkers.

	Verbal fluency				MDa	95%	CI	p value
	MD crude	95%	CI	p value				
Exposure indices								
Source distance from IES (≤1.5 *vs* 1.5+ km)								
Letter P	−1.54	−2.39	−0.69	<0.001	−1.51	−2.40	−0.62	0.001
Letter M	−1.42	−2.17	−0.66	<0.001	−1.47	−2.26	−0.67	<0.001
Letter R	−1.58	−2.39	−0.78	<0.001	−1.60	−2.45	−0.74	<0.001
PM personal samplers (ng/m^3)								
Coarse fraction (PM10–2.5)								
Bioaccessible (13.62+ vs ≤ 13.61 ng/m^3)								
Letter P	−1.26	−2.13	−0.40	0.005	−1.17	−2.07	−0.28	0.010
Letter M	−0.86	−1.64	−0.09	0.030	−0.83	−1.65	−0.02	0.044
Letter R	−1.55	−2.36	−0.74	<0.001	−1.52	−2.36	−0.67	0.001
Non-bioaccessible (3.39+ vs ≤ 3.38 ng/m^3)								
Letter P	−0.22	−1.11	0.68	0.634	−0.10	−1.00	0.79	0.819
Letter M	−0.31	−1.10	0.48	0.442	−0.26	−1.07	0.54	0.518
Letter R	−1.09	−1.93	−0.26	0.011	−1.03	−1.88	−0.18	0.018
Total (Bio + non-bio) (16.48+ vs ≤ 16.47 ng/m^3)								
Letter P	−1.35	−2.22	−0.49	0.002	−1.25	−2.14	−0.36	0.006
Letter M	−0.89	−1.67	−0.12	0.024	−0.87	−1.68	−0.06	0.036
Letter R	−1.49	−2.31	−0.68	<0.001	−1.45	−2.29	−0.60	0.001
Fine fraction (PM2.5)								
Bioaccessible (17.06+ vs ≤ 17.05 ng/m^3)								
Letter P	−1.14	−2.01	−0.27	0.011	−1.07	−1.95	−0.19	0.018
Letter M	−0.98	−1.76	−0.21	0.013	−0.95	−1.75	−0.16	0.019
Letter R	−1.12	−1.96	−0.29	0.009	−1.06	−1.91	−0.21	0.015
Non-bioaccessible (5.81+ vs ≤ 5.80 ng/m^3)								
Letter P	−0.43	−1.32	0.46	0.340	−0.27	−1.17	0.64	0.558
Letter M	−0.31	−1.10	0.48	0.442	−0.25	−1.07	0.56	0.537
Letter R	−0.48	−1.33	0.37	0.269	−0.37	−1.25	0.50	0.400
Total (Bio + non-bio) (25.01+ vs ≤ 25.00 ng/m^3)								
Letter P	−1.32	−2.19	−0.46	0.003	−1.21	−2.10	−0.32	0.008
Letter M	−1.08	−1.84	−0.31	0.006	−1.06	−1.86	−0.26	0.010
Letter R	−1.15	−1.98	−0.32	0.007	−1.08	−1.94	−0.21	0.015
All fractions (PM10) (43.88+ vs ≤ 43.87 ng/m^3)								
Letter P	−1.14	−2.01	−0.27	0.011	−0.97	−1.87	−0.07	0.034
Letter M	−0.83	−1.61	−0.05	0.036	−0.81	−1.62	0.00	0.051
Letter R	−1.22	−2.04	−0.39	0.004	−1.14	−2.01	−0.28	0.010
Biomarkers								
Blood Mn (9.59+ vs ≤ 9.58 μg/L)								
Letter P	0.80	−0.08	1.68	0.075	0.66	−0.24	1.57	0.149
Letter M	0.52	−0.26	1.31	0.190	0.54	−0.27	1.36	0.191
Letter R	0.42	−0.44	1.27	0.336	0.39	−0.49	1.27	0.383
Scalp hair Mn (185.31+ vs ≤ 185.30 ng/g)								
Letter P	−0.54	−1.43	0.35	0.232	−0.46	−1.37	0.44	0.313
Letter M	−0.34	−1.16	0.49	0.421	−0.38	−1.23	0.47	0.373
Letter R	−0.53	−1.40	0.34	0.231	−0.56	−1.45	0.34	0.220
Fingernails Mn (555.28+ vs ≤ 555.28 ng/g)								
Letter P	−0.50	−1.47	0.47	0.308	−0.40	−1.42	0.62	0.440
Letter M	−0.60	−1.46	0.25	0.164	−0.58	−1.49	0.33	0.208
Letter R	−0.57	−1.48	0.34	0.216	−0.54	−1.51	0.43	0.270

IES = Industrial Emission Source. MD = Mean Difference. MDa = adjusted for age, sex and study level. *A negative MD indicates worse cognitive function in exposed to higher Mn levels.

Regarding biomarkers, negative nonstatistically significant crude and adjusted MDs were obtained for Mn fingernails levels and mixed nonsignificant results were obtained for scalp hair and blood Mn levels with negative MDs for the ROCF Copy and positive MDs for the Delayed part. In accordance, higher ORs were obtained for the fingernail Mn levels and the Copy part: crude OR = 2.16; 95%CI (1.03 to 4.54) (see Tables S27 to S29).

In terms of correlations, statistically significant coefficients were obtained only for the Non-bioaccessible fine fraction and the ROCF Delayed part (rho = −0.182, p = 0.038) and fingernail levels and ROCF Copy Part (rho = −0.213, p = 0.022) (see Table S25).

4. Discussion

Our results support a worse cognitive function as determined by the Stroop Color Word test in relation to the proximity to the IES, and several PEMs determinations. In terms of biomarkers, Mn fingernail levels, and to a lesser extent Mn hair levels, were also associated with poorer cognitive function according to this test. Each of the tests used assessed different domains of cognitive function, in particular the Stroop Color Word test measures mainly executive function. To the best of our knowledge, quantitative results in this test have only been reported by Bowler et al. (2015), where negative correlations were observed between all three parts of the test and Mn airborne levels indirectly estimated through a dispersion model, although these correlations only reached statistical significance for the second and third part of the test (Color and Color&Word). In the articles published by Beuter et al. (1999), Mergler et al. (1999), (from the same study), and Kornblith et al. (2018), the use of this test is mentioned but no quantitative results are reported.

Our results also support a worse cognitive function in the Language (verbal fluency) domain as determined through the Verbal Fluency test (letters P, M, R); especially in relation to the proximity to the IES and the bioaccessible fractions of PEMs determinations, and to a lesser extent, in relation to fingernails and hair Mn levels. Our results support the inclusion

Table 4
Mean differences for TMT according to Mn exposure indices and biomarkers.

	TMT				MDa	95%	CI	p value
	MD crude	95%	CI	p value				
Exposure indices								
Source distance from IES (≤1.5 *vs* 1.5+ km)								
TMT-A	−1.00	−1.98	−0.02	0.045	−1.32	−2.34	−0.31	0.011
TMT-B	−1.14	−1.92	−0.36	0.005	−0.97	−1.78	−0.15	0.021
PM personal samplers (ng/m^3)								
Coarse fraction (PM10–2.5)								
Bioaccessible (13.62+ vs ≤ 13.61 ng/m^3)								
TMT-A	−0.32	−1.31	0.67	0.520	−0.52	−1.54	0.51	0.323
TMT-B	−0.68	−1.48	0.12	0.096	−0.53	−1.35	0.28	0.199
Non-bioaccessible (3.39+ vs ≤ 3.38 ng/m^3)								
TMT-A	−0.20	−1.19	0.79	0.691	−0.31	−1.32	0.70	0.540
TMT-B	0.12	−0.68	0.93	0.763	0.25	−0.56	1.05	0.546
Total (Bio + Non-Bio) (16.48+ vs ≤ 16.47 ng/m^3)								
TMT-A	−0.32	−1.31	0.67	0.520	−0.51	−1.53	0.52	0.331
TMT-B	−0.52	−1.32	0.28	0.199	−0.39	−1.20	0.43	0.354
Fine fraction (PM2.5)								
Bioaccessible (17.06+ vs ≤ 17.05 ng/ m^3)								
TMT-A	−0.66	−1.65	0.32	0.187	−0.77	−1.78	0.23	0.128
TMT-B	−0.65	−1.45	0.15	0.112	−0.56	−1.37	0.24	0.167
Non-bioaccessible (5.81+ vs ≤ 5.80 ng/m^3)								
TMT-A	0.26	−0.73	1.25	0.603	0.18	−0.84	1.19	0.732
TMT-B	−0.37	−1.17	0.44	0.365	−0.31	−1.12	0.51	0.457
Total (Bio + Non-Bio) (25.01 + vs ≤ 25.00 ng/m3)								
TMT-A	−0.57	−1.56	0.42	0.256	−0.75	−1.77	0.27	0.149
TMT-B	−0.62	−1.42	0.18	0.130	−0.51	−1.33	0.30	0.215
All fractions (PM10) (43.88+ vs ≤ 43.87 ng/m^3)								
TMT-A	−0.42	−1.41	0.57	0.408	−0.57	−1.59	0.45	0.274
TMT-B	−0.52	−1.32	0.28	0.199	−0.45	−1.27	0.37	0.276
Biomarkers								
Blood Mn (9.59+ vs ≤ 9.58 μg/L)								
TMT-A	0.14	−0.85	1.13	0.783	0.25	−0.77	1.28	0.625
TMT-B	0.68	−0.12	1.48	0.096	0.61	−0.20	1.43	0.138
Scalp hair Mn (185.31+ vs ≤ 185.30 ng/g)								
TMT-A	−0.35	−1.38	0.68	0.504	−0.28	−1.35	0.79	0.602
TMT-B	−0.05	−0.89	0.78	0.903	−0.23	−1.07	0.61	0.587
Fingernails Mn (555.28+ vs ≤ 555.28 ng/g)								
TMT-A	−0.67	−1.71	0.36	0.200	−0.99	−2.08	0.10	0.075
TMT-B	−1.00	−1.83	−0.17	0.018	−0.75	−1.61	0.12	0.091

IES = Industrial Emission Source. MD = Mean Difference. MDa = adjusted for age, sex and study level. *A negative MD indicates worse cognitive function in exposed to higher Mn levels.

of this test in future epidemiological studies in order to compare our results, since no published studies have included it up to our knowledge.

The TMT (A and B) has been used in several epidemiological studies (Bowler et al., 2015; Lucchini et al., 2014; Rafiee et al., 2020; Santos-Burgoa et al., 2001; Viana et al., 2014). This test measures mainly attention. In our study, significant associations were found for both parts in relation to proximity to the IES and of lower magnitude for the bioaccessible PEMs fractions; whereas they were found more specifically only for part B in relation to fingernails and hair Mn levels. Bowler et al. (2015) found a non-significant negative correlation between part B and airborne Mn levels from two US towns with high industrial airborne Mn exposure (Marietta and East Liverpool). Viana et al. (2014) found negative correlations for both part A and B, especially in relation to Mn levels in fingernails, as well as negative MDs for part B, when comparing scores from two communities in Brazil located approximately 1.5 and 2.5 km away from a ferromanganese alloy plant. Lucchini et al. (2014) found negative MDs on both TMT parts when comparing results from two regions, an industrial one (Valcamonica, n = 153) close to a former Mn alloy plant exposed to significantly higher environmental levels than the reference region (Garda Lake n = 102). The study by Santos-Burgoa et al. (2001) with a study population consisting of two communities within a Mn mining district in central Mexico: one located 2 km from a primary Mn ore refinery plant and the other located further away (25 km from the source), found a statistically non-significant increased adjusted risk ratio when blood Mn was above the 75th percentile, in comparison to the lowest population 25th percentile level. In the study published by Rafiee et al., (2020) with Iranian volunteers in which their hair Mn levels were determined, statistically significant adjusted linear regression coefficients were obtained for both parts of the TMT. The results of these studies would support our findings. As in the case of the Stroop test, the articles published by Beuter et al. (1999), Mergler et al. (1999) and Kornblith et al. (2018) also mention the use of the TMT but do not report quantitative results.

We also obtained statistically significant adjusted MDs in terms of proximity to the IES, bioaccessible coarse fraction, fingernails and hair Mn levels in relation to Digit Span test results (Forward and Backward). This test assesses attention and working memory as parts of the executive function. Negative correlations using this test for airborne Mn levels (Bowler et al., 2015) and biomarkers (hair, nails and saliva) (Viana et al., 2014) have been reported. Finally, Viana et al. (2014) and Lucchini et al. (2014) found MDs close to null or positives (against our hypothesis) of very slight magnitude when comparing results in this test between the populations described above for the TMT. Santos-Burgoa et al. (2001) also reported positive nonsignificant adjusted risk ratios for this test when comparing volunteers with higher and lower blood Mn levels. Also in México, Solís-Vivanco et al. (2009) found positive associations in the form of statistically significant Odds Ratios: OR = 1.75; 95%CI (1.01 to 3.06) for the air Mn cut-off point of 0.1 μg/m^3. Again, Beuter et al. (1999) and Mergler et al. (1999) mention the use of this test without reporting results.

In relation to ROCF, which mainly assesses memory and constructive praxia (visual perception and delayed visuospatial memory), the evidence of association was lower, with some significant associations mainly for the ROCF Copy part in terms of proximity to the IES and fingernails Mn levels; and for the Delayed part in terms of the non-bioaccessible fine fraction Mn levels. To our knowledge, only Bowler et al. (2015) published results for this test, finding negative correlations (significant for the Delayed part), whereas Kornblith et al. (2018) mentioned the use of this test without reporting results.

Regarding confounding bias, as expected, raw cognitive tests scores differed according to age and educational level, so comparisons between cognitive test results and Mn levels are presented adapted to NEURONORMA, this is, adapted to age and educational level and standardized to a scale with a homogeneous range of 2–18 (Peña-Casanova et al., 2009). In several of the cognitive tests used (Stroop Color Word, Verbal Fluency and ROCF tests), even though the NEURONORMA standardization, statistically significant differences were observed for age or study level when comparing basal characteristics of study population as a function of cognitive test results, being able to potentially create a residual confounding. For this reason they were predefined with sex as confounding variables.

A high number of comparisons have been made, especially regarding PEMs results, in the context of an exploratory approach differentiating between fine & coarse and bioaccessible & non - bioaccessible fractions, with the possibility of obtain spurious associations due to chance by a multiple-testing problem. The fact that our results are in line with those of published studies would also support that they are not due to chance. In addition, concordance between our results in our three different analytical strategies (Spearman correlations, MDs and ORs) would support the internal validity of results.

As our study has a cross-sectional design, without longitudinal data on both the Mn exposure and the cognitive function determinations. However most of our participants have a number of years of residence enough to establish a long-term exposure covering the potential latency period between exposure and the onset of effects on cognitive function. Despite the lack of cohort studies on the relationship between environmental Mn airborne exposure and cognitive function in adults, an increasing number of longitudinal studies is available evaluating Mn exposure during pregnancy, childhood and at youth ages. Classically mixed results have been obtained from these studies: a) adverse associations have been reported in some of them (Freire et al., 2018; Lin et al., 2013; Yang et al., 2014); b) no associations have been reported in others (Andiarena et al., 2017; Claus Henn et al., 2017; Gunier et al., 2015; Soler-Blasco et al., 2020); and c) beneficial associations between prenatal Mn exposure and

Table 5
Mean differences for digit span test according to Mn exposure indices and biomarkers.

	Digit Span				MDa	95%	CI	p value
	MD crude	95%	CI	p value				
Exposure indices								
Source distance from IES (≤1.5 *vs* 1.5+ km)								
Digit Span Forward	−1.05	−1.92	−0.17	0.020	−0.97	−1.90	−0.04	0.041
Digit Span Backward	−1.22	−1.98	−0.46	0.002	−1.29	−2.08	−0.49	0.002
PM personal samplers (ng/m^3)								
Coarse fraction (PM10–2.5)								
Bioaccessible (13.62+ vs ≤ 13.61 ng/m^3)								
Digit Span Forward	−0.52	−1.42	0.37	0.248	−0.39	−1.32	0.54	0.403
Digit Span Backward	−1.03	−1.80	−0.26	0.009	−1.02	−1.81	−0.22	0.013
Non-bioaccessible (3.39+ vs ≤ 3.38 ng/m^3)								
Digit Span Forward	−0.92	−1.81	−0.04	0.040	−0.84	−1.74	0.06	0.066
Digit Span Backward	−0.08	−0.87	0.71	0.847	−0.03	−0.83	0.77	0.943
Total (Bio + Non-Bio) (16.48+ vs ≤ 16.47 ng/m^3)								
Digit Span Forward	−0.52	−1.42	0.37	0.248	−0.39	−1.32	0.54	0.408
Digit Span Backward	−0.82	−1.59	−0.04	0.040	−0.78	−1.58	0.03	0.058
Fine fraction (PM2.5)								
Bioaccessible (17.06+ vs ≤ 17.05 ng/m^3)								
Digit Span Forward	−0.25	−1.14	0.65	0.588	−0.15	−1.07	0.76	0.742
Digit Span Backward	−0.35	−1.14	0.43	0.375	−0.30	−1.10	0.50	0.464
Non-bioaccessible (5.81+ vs ≤ 5.80 ng/m^3)								
Digit Span Forward	−0.71	−1.60	0.18	0.117	−0.60	−1.51	0.31	0.195
Digit Span Backward	−0.20	−0.99	0.59	0.616	−0.11	−0.91	0.70	0.794
Total (Bio + Non-Bio) (25.01+ vs ≤ 25.00 ng/m^3)								
Digit Span Forward	−0.28	−1.17	0.62	0.542	−0.13	−1.06	0.80	0.786
Digit Span Backward	−0.38	−1.17	0.40	0.335	−0.30	−1.11	0.51	0.465
All fractions (PM10) (43.88+ vs ≤ 43.87 ng/m^3)								
Digit Span Forward	−0.46	−1.35	0.43	0.309	−0.32	−1.24	0.61	0.499
Digit Span Backward	−0.35	−1.14	0.43	0.375	−0.26	−1.07	0.55	0.530
Biomarkers								
Blood Mn (9.59+ vs ≤ 9.58 μg/L)								
Digit Span Forward	−0.03	−0.93	0.87	0.946	−0.14	−1.06	0.79	0.768
Digit Span Backward	0.32	−0.46	1.11	0.418	0.33	−0.48	1.14	0.419
Scalp hair Mn (185.31+ vs ≤ 185.30 ng/g)								
Digit Span Forward	−1.03	−1.92	−0.13	0.025	−1.14	−2.06	−0.21	0.016
Digit Span Backward	−0.67	−1.48	0.14	0.104	−0.63	−1.46	0.20	0.137
Fingernails Mn (555.28+ vs ≤ 555.28 ng/g)								
Digit Span Forward	−1.64	−2.55	−0.73	0.001	−1.60	−2.57	−0.64	0.001
Digit Span Backward	−1.26	−2.06	−0.46	0.002	−1.45	−2.29	−0.61	0.001

IES = Industrial Emission Source. MD = Mean Difference. MDa = adjusted for age, sex and study level. *A negative MD indicates worse cognitive function in exposed to higher Mn levels.

neurodevelopment have been even reported (Mora et al., 2015). These mixed results can be explained by the heterogeneity in the methodology of the published studies (Bauer et al., 2020b; Liu et al., 2020) and by the fact that Mn is an essential nutrient required for growth and neurodevelopment. Evidence from the latest studies suggests that exposure timing is critical when evaluating Mn associations between Mn and cognitive function. Whereas higher prenatal Mn seems beneficial even for adolescent cognitive function; later time windows such as childhood Mn exposure seem to be associated to poorer cognitive function on visuospatial ability, working memory, attention and problem-solving domains (Bauer et al., 2021; Irizar et al., 2021). In any case, further follow-up studies are clearly needed in adults to deep in the cognitive function effects derived from the chronic environmental airborne Mn exposure. The clinical relevance of possible poorer cognitive function results of small magnitude must also be elucidated.

On the other hand, with respect to the use of the bioaccessible Mn concentration instead of its total content, only results from the Verbal Fluency tests battery led to statistically significant MDs of greater magnitude for the bioaccessible fractions. Fort the rest of the tests, our results show no clear differences when analyzing bioaccessible and non-bioaccessible fractions separately: for TMT parts A and B only slightly higher MDs for the bioaccessible fractions were obtained. Negative MDs of greater magnitude were obtained only for the bioaccessible fine fraction and the ROCF Copy part without reaching statistical significance, whereas for the ROCF Delayed part higher and significant negative MDs were obtained for the non-bioaccessible fine fraction. For the Stroop Color Word test and the Digit Span test, only MDs of higher magnitude were obtained in some parts of the tests. The OR approach was in concordance with MDs approach. In general, correlation approach was also in concordance with higher Spearman coefficients for the bioaccessible fraction. Although the potential health effects from the exposure to PM-bound Mn might depend to a greater extent on its solubility in the human body, this solubility being influenced by the chemical speciation of these metal(loid)s, the size and shape of particles and the chemical composition of the biological fluid (Expósito et al., 2021a; Hernández-Pellón et al., 2018; Kelly and Fussell, 2012), the results shown here for the cognitive constructs studied suggest a more complex relationship that needs further investigation.

In addition, the fact that the fine fraction has a greater penetration into the smaller diameter airways presupposed greater differences respect to coarse fractions, regardless of their bioaccessibility. In this respect, for some of the tests such as the ROCF, larger effect sizes have been found for the fine fraction. However, for other tests such as the Verbal Fluency or the TMT no major differences were found. Contrary to predefined, for the Stroop Color Word test and the Digit Span, although there are no large differences either, the effect sizes tend to be more favorable for the coarse fraction. It would be an aspect that needs further investigation.

Regarding biomarkers, as outlined in the introduction section, the main exposure pathway for our Mn levels in biomarkers would be the inhalation route (Fernández-Olmo et al., 2021). In epidemiologic studies a biomarker of exposure should have a predictive potential for adverse health effects (Democophes, 2012; Fernández-Olmo et al., 2021; Viana et al., 2014; Zheng et al., 2011;), ideally with a dose-response pattern. Our three selected biomarkers (blood, hair and nails) aimed to estimate short-, medium- and

long-term exposure respectively (Aschner et al., 2007; Haynes et al., 2015; Michalke and Fernsebner, 2014; Ntihabose et al., 2018; Viana et al., 2014). Our results did not support an association between higher Blood Mn levels and worse cognitive function, with contrary associations in some tests. In addition, no correlation between source distance from the IES and Blood Mn levels was found. This could be interpreted in terms of the inadequacy of blood as a biomarker of exposure to Mn in epidemiologic studies (Roth, 2006; Zheng et al., 2011). However, our homogeneous results for fingernails in relation to cognitive function test results and the negative significant correlation with source distance from the IES, support its consideration as the biomarker of choice, in agreement with published studies (Coetzee et al., 2016; Ntihabose et al., 2018; Viana et al., 2014). With respect to hair, in general, lower negative effects were obtained, probably because it represents shorter exposure periods than nails (Bouchard et al., 2011; Coetzee et al., 2016; Viana et al., 2014; Haynes et al., 2015).

As conclusion, our results support an association between poorer cognitive function and environmental airborne Mn exposure in adults, especially in terms of proximity to IES, being supported also by different PEMs determinations. Depending on the test, the results most favorable to our hypothesis in terms of worse cognitive function were obtained for the Stroop Color Word, Verbal Fluency test, TMT and Digit Span test, respectively. For the ROCF the evidence was lower. Regarding biomarkers, Mn fingernails and to a lesser extent Mn hair levels, were associated with worse cognitive function outcomes. Our homogeneous and favorable results for fingernail Mn levels support the consideration of this biomarker as a useful biomarker of exposure to Mn in epidemiological studies.

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CRediT authorship contribution statement

M.S. and I.F.-O: Conceptualization, Methodology, Writing-review & editing, Supervision and Funding acquisition. L.R.-A: Investigation, Writing -original draft and Formal analysis. B.M, A.E., A.P. and M.G.-M: Investigation and Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.152940.

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