Neurotoxicology 87 (2021) 195–207

Contents lists available at ScienceDirect

Neurotoxicology

journal homepage: www.elsevier.com/locate/neuro

Biomonitoring and bioaccessibility of environmental airborne manganese in relation to motor function in a healthy adult population

Laura Ruiz-Azcona [a], Bohdana Markiv [b], Andrea Expósito [b], Isabel González-Aramburu [c], María Sierra [c], Ignacio Fernández-Olmo [b], Miguel Santibáñez [a,d,*]

[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, Hospital Universitario Marqués de Valdecilla (HUMV-IDIVAL), Santander, Spain
[d] Nursing Research Group, IDIVAL, Calle Cardenal Herrera Oria s/n, 39011, Santander, Cantabria, Spain

ARTICLE INFO

Edited by Dr. P. Lein and Dr. R. Westerink

Keywords:
Environmental exposure
Manganese
Motor function
Biomarker
PM

ABSTRACT

Background/aim: Santander, the capital of Cantabria, Spain (172,000 inhabitants) is 7 km from an industrial emission source (IES) of Mn located in a 10,000 inhabitants town (Maliaño) (annual air Mn arithmetic mean = 231.8 ng/m^3; reference WHO guideline = 150 ng/m^3). Our objective was to compare the motor function of adult healthy volunteers living in both places.

Methods: Cross-sectional study analyzing 130 consecutive participants. Exposure to Mn was assessed in terms of source distance from the IES, by Personal Environmental Monitors (PEMs) carried for 24 h by participants consisting of a portable impactor connected to a personal pump, and by biomarkers (blood, hair and fingernails). The impactor allowed the separation of fine (PM2.5) and coarse (PM10-2.5) particles and for each particle size in-vitro bioaccessibility tests with biologically active fluids were performed to separate the soluble (bioaccessible) from the insoluble (non-bioaccessible) fraction. Mean Differences (MDs) adjusted for age, sex, and study level, were obtained for motor function tests results.

Results: Regarding Grooved Pegboard, overall mean time to complete the test was 59.31 and 65.27 seconds (Standard Deviation = 10.11 and 11.69) for dominant and nondominant hands respectively. Statistically significant higher times (indicating worse function) were observed when living near the IES in both hands but MDs of only 1.22 and 2.05 seconds were obtained after adjusting for the predefined confounders (p = 0.373 and 0.221 respectively). Regarding Mn levels in their PEMs (in both bioaccessible and non-bioaccessible coarse&fine fractions) higher times were computed in participants with higher levels for the bioaccessible-fine fraction, with a MD that diminished but still yielded statistical significance after controlling for confounding: adjusted MD = 3.01 more seconds; 95%CI (0.44–5.38), p = 0.022. Poorer results were also observed for fingernails levels. Regarding Finger Tapping Test, no statistically significant differences were found with the exception of Mn fingernails levels.

Conclusions: Our results suggest poorer motor function as assessed by Grooved Pegboard test in relation to "proximity to IES", "bioaccessible-fine fraction as determined by PEMs and "Mn fingernails levels". However, our findings were affected by confounding, and only the adjusted MD for the Mn bioaccessible-fine fraction remained of sufficient magnitude to maintain statistical significance.

1. Introduction

Manganese (Mn) is a trace element, and therefore an essential nutrient found in the human body (Chen et al., 2018). However, neurological and neuropsychological negative health effects have been described in relation to high levels of occupational Mn exposure (Lucchini et al., 2009; Williams ATSDR, 2012), mainly in the context of Mn ferroalloys plants and welders (Bowler et al., 2006a, b; Lucchini et al., 1999; Mergler et al., 1994), Mn ore mines (Dlamini et al., 2020), and Mn ore processing plants (Roels et al., 1987).

* 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).

https://doi.org/10.1016/j.neuro.2021.10.005
Received 25 June 2021; Received in revised form 14 October 2021; Accepted 14 October 2021
Available online 19 October 2021

Although most health studies on Mn overexposure regard to occupational exposure, a few epidemiological studies have also been published in recent decades with regard to environmental airborne exposure, as reviewed by Fernández-Olmo et al. (2020), conducted in areas near the industrial emission sources (IES) of Mn mentioned above, this is, Mn ferroalloys plants (Bowler et al., 2012, 2015, 2016; Kim et al., 2011; Lucchini et al., 2014; Standridge et al., 2008; Viana et al., 2014), Mn ore mines (Guarneros et al., 2013), and Mn ore processing plants (Bowler et al., 2012, 2015). Neurological and neuropsychological outcomes can be classified in these studies into cognitive, motor, olfactory and emotional function and mood (Fernández-Olmo et al., 2020).

In relation to motor function and environmental airborne Mn exposure, a recent meta-analysis by Ruiz-Azcona et al. (2021) identified only eight studies (Bowler et al., 2012, 2016; Guarneros et al., 2013; Kim et al., 2011; Lucchini et al., 2014; Mergler et al., 1999; Standridge et al., 2008; Viana et al., 2014). In these studies, the exposed populations were located in Southwest Quebec, Canada, where a former Mn alloy production plant existed (Mergler et al., 1999); Marietta and East Liverpool, Ohio, two towns near a ferromanganese refinery and a Mn ore processing plant in the United States (Bowler et al., 2012, 2016; Kim et al., 2011; Standridge et al., 2008); a Mn mining district living <1 km from a manganese processing plant (Tolago/Chiconcoac), in the Molango district in the High Sierra of the central Mexican state of Hidalgo (Guarneros et al., 2013); Valcamonica, Italy, an industrial region close to former Mn alloy plants (Lucchini et al., 2014); Cotegipe, Simões Filho, Bahia, Brazil a small community situated at an approximate distance of 1.5 km from a ferromanganese alloy plant (Viana et al., 2014). Most of these areas may be considered as low-density populated.

In one hand, means of motor function tests scores in these exposed populations were compared with populations residing in locations farther away from the Mn emission sources in seven studies (Bowler et al., 2012; Guarneros et al., 2013; Kim et al., 2011; Lucchini et al., 2014; Mergler et al., 1999; Standridge et al., 2008; Viana et al., 2014). On the other hand, correlation between Mn levels in the exposed populations and motor function tests scores was analyzed in three studies (Bowler et al., 2016; Standridge et al., 2008; Viana et al., 2014). The meta-analysis showed a statistically significant worse motor function in exposed, and a negative correlation (the higher the Mn levels, the poorer scores).

Airborne exposure to trace metal(loid)s near IES of such pollutants may be assessed by measuring their concentration in particulate matter (PM) filters using stationary or personal samplers. Stationary PM samplers have been using for years to determine metals in ambient PM, but its spatial representativeness is limited (Fulk et al., 2016).

PM personal samplers, also known as Personal Environmental Monitors (PEM), could solved this limitation but their use is still not widely implemented (Graney et al., 2004; Haynes et al., 2012; Lucchini et al., 2012; Pollitt et al., 2016; Solís-Vivanco et al., 2009). Finally, although PEMs are used in some studies, they only consider the total content of metal instead its soluble fraction in a biologically active fluid, *i.e.* its bioaccessibility (Expósito et al., 2021). Different surrogate fluids may be used in such bioaccessibility assays, mainly artificial lung fluids to assess the inhalation route of exposure (Kastury et al., 2017, 2018; Mbengue et al., 2015; Mukhtar and Limbeck, 2013) and gastric fluids to assess the oral route (Denys et al., 2012; European Pharmacopoeia, 2010; USEPA, 2007). It is relevant to differentiate between the bioaccessible and non-bioaccessible fractions in PM, since the soluble (bioaccessible) fraction of PM-bound trace metal(loid)s instead of total content may better represent the exposure risk of such pollutants in humans (Hernández-Pellón et al., 2018; Weggeberg et al., 2019). Metal bioaccessibility in a given fluid mainly depends on the chemical speciation of such metal, which also depends on its source. Thus, metal bioaccessibility differs between urban, industrial and rural areas (Mukherjee and Agrawal, 2017); for example, Mn inhalation bioaccessibility near industrial sources was found to be higher than in a closer urban area, thus leading to a higher potential risk (Hernández-Pellón et al., 2018). With respect to specific studies on the relationship between environmental airborne Mn exposure and motor function, none of the eight studies included in the meta-analysis have evaluated Mn exposure by using PEMs and very few of them quantified airborne exposure, indirectly by modelling (Bowler et al., 2012, 2016; Kim et al., 2011) or directly by stationary air Mn evaluation (Lucchini et al., 2014).

In addition to personal samplers, the analysis of specific biomarkers can also account for the spatial variability of the exposure. A biomarker in an epidemiological context, should be able to characterize and differentiate exposed and non-exposed groups, as well as to predict health disorders, as a result of short- or long-term exposure (Viana et al., 2014; Zheng et al., 2011). Most of the studies included in the meta-analysis on motor function have used biomarkers to determine Mn exposure (Bowler et al., 2012; Guarneros et al., 2013; Kim et al., 2011; Lucchini et al., 2014; Mergler et al., 1999; Standridge et al., 2008; Viana et al., 2014), "whole blood" being the most biological sample used (Bowler et al., 2012; Kim et al., 2011; Lucchini et al., 2014; Mergler et al., 1999; Standridge et al., 2008) followed by scalp and/or axillary hair (Guarneros et al., 2013; Standridge et al., 2008; Viana et al., 2014), urine (Lucchini et al., 2014) and fingernails and saliva (Viana et al., 2014). Since each biomarker has strengths and weaknesses, *i.e.* blood aims to estimate short term exposure, hair aims to estimate an exposure of 1–6 months, whereas nails allow to estimate low-level chronic exposure (Fernández-Olmo et al., 2020; Ntihabose et al., 2018; Haynes et al., 2015; Viana et al., 2014), most of studies use more than one biomarker.

Santander, the capital of Cantabria, Northern Spain with a high population density (172,000 inhabitants) is only 7 km from an important IES of Mn (a ferromanganese alloy plant) located in a 10,000 inhabitants town (Maliaño), leading to relatively high levels of airborne Mn in comparison with the annual reference guideline given by WHO (150 ng/m^3). In this context, an annual arithmetic mean of 231.8 ng/m^3 was measured in downtown Maliaño in 2015 (Hernández-Pellón and Fernández-Olmo, 2019a); higher monthly values were reported in sites located closer to the IES, a cultural center (La Vidriera), mean of 721.9 ng/m^3, and a primary school (Juan de Herrera), mean of 713.9 ng/m^3 (Hernández-Pellón and Fernández-Olmo, 2019b). Even in Santander, an arithmetic mean of 60.8 ng/m^3 was reported in 2015 (Hernández-Pellón and Fernández-Olmo, 2019a), which exceeds the Reference Concentration (RfC) given by US EPA (50 ng/m^3).

Therefore, the objective of this study was to analyze the impact of environmental Mn exposure on motor 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 study was carried out in Santander Bay (Cantabria, northern Spain) where there is a ferromanganese alloy plant that produces more than 100 kt of ferromanganese and silicomanganese, annually. The area of study has been described in detail elsewhere (Arruti et al., 2010, 2011a, b; Hernández-Pellón and Fernández-Olmo, 2019a, b) as well as in the introduction section.

Participants were recruited from November 2019 to November 2020, among a list of 165 potential volunteers obtained by three different strategies: a) At the Researchers' Night organised by the University of Cantabria in the 2018 and 2019 sessions in Santander, the project was publicised and a list of potential volunteers was obtained. b) An information day organised by Camargo Town Council in the La Vidriera Cultural Centre (Maliaño), attended by representatives of the different Neighbourhood Associations of Maliaño. c) Diffusion by means of

informative posters, invitation letters and pamphlets in the Hospital Universitario Marqués de Valdecilla (HUMV), Primary Care Centres, University of Cantabria and Maliaño Neighbourhood Associations. Fig. S1 shows the flow chart to obtain the final study population: seventeen out of the 165 preselected candidates could not be contacted by phone. Eight people declined to participate when contacting. The remaining 140 participants completed a brief questionnaire by phone to verify the inclusion criteria of age (between 18–75 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.
- Residence criterion 2: ≥ 10 years of residence during the last 15 years located up to 10 km away from the IES.
- 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.

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.
- 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.
- Active alcohol consumption and/or other psychotropic drugs.
- Lack of understanding of the Spanish language for the purpose of completing the tests.

Finally, among the 140 participants, 130 met the criteria to participate in the study. This population was divided into two groups: (1) highly exposed (n = 65): participants living or working within ≤1.5 km from the IES, *i.e.* those living in Maliaño, where the main Mn source is located, and (2) moderately exposed (n = 65): participants living or working between >1.5 to ≤10 km from the IES, *i.e.* those living outside Maliaño, mainly in the city of Santander.

One hundred and twenty-three of the 130 volunteers (94.6 %) met the first residence criterion (≥1 last year ≤ 10 km from the Mn source); 118 (90.8 %) the second (≥10 years in the last 15 years residing ≤10 km from the IES) and 128 (98.5 %) the third (≥10 years in the last 15 years with workplace ≤10 km from the IES). Table S1 presents the cumulative compliance with the residence criteria: 89.2 % of the sample met both residency criteria at the same time. 6.9 % met one of them, so 96.2 % of the sample met at least 1 of the residency criteria. The remaining n = 5 (3.8 %) met only the workplace criterion.

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. Data collection. Motor function tests

Firstly, an individual first appointment was scheduled with each of the 130 volunteers at a Testing room of the University of Cantabria that met the privacy needs for sensitive evaluations. During each of the appointments, the sequence of steps was as follows:

1) Signature of the informed written consent form. 2) Personal interview using a structured questionnaire with socio-demographic data, place of residence and work and years residing, work history, medical history, pharmacological treatments, tobacco and alcohol consumption, psychotropic drugs use and dietary habits including daily intake of Mn-rich foods and Mn food supplements. 3) Cognitive and motor function tests battery (always in the same order) provided no use of alcohol and/or other psychotropic drugs <6 h before testing (no active consumption). 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 HUMV.

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.

The standardized motor function test battery included three tests to evaluate motor function. "Eye-hand coordination and motor speed", "self-directed manual speed" and grip strength were measured using the Finger Tapping Test (FTT), the Grooved Pegboard (GP) and the Hand Dynamometer, respectively (Strauss et al., 2006).

The Finger Tapping test (Lezak, 2004) is a measure of psychomotor speed requiring examinees to tap a lever with their index finger as fast as possible in 10 seconds for the dominant (dom) and nondominant (nondom) hands separately. The score is the number of taps for each hand, recorded by a counter (WPS electronic Tapping Test).

Self-directed manual speed and tactile manipulative ability was assessed with the Grooved Pegboard test (Bornstein, 1986) which requires speeded rotation and insertion of pegs with ridges on them in a 5 × 5 matrix of holes with the dom and nondom hands. Model 32025 of Lafayette Instrument Company was used. The score for each hand is based on the time (seconds) required to complete the task.

Grip strength was assessed in kilograms with the use of the Dynamometer T.K.K. 5401 Grip-D, Takei, Tokyo, Japan (Oteo et al., 2015), which participants were asked to squeeze twice for each hand. The highest scores from the two measurements with each hand were selected.

2.3. Personal exposure to airborne Mn

Inhalation personal exposure to size segregated PM was determined using a portable impactor (SKC PMI coarse) connected to a personal pump (SKC Aircheck XR5000) that operated at a flow rate of 3 lpm. The impactor allows the separation of the fine (PM2.5) and coarse (PM10-2.5) modes, collecting them on 37 and 25 mm PTFE membrane filters, respectively.

Both filters were analyzed in a two-step procedure according to the method developed by Expósito et al. (2021). First, in-vitro bioaccessibility test was carried out by extracting each filter with 10 mL of the selected leaching agent for 24 h (PM2.5) or 1 h (PM10-2.5) in an end-over-end rotation incubator system at 30 rpm and 37 °C. After the leaching test, samples were centrifuged, and the supernatants were filtered. According to this procedure, gastric fluid and Artificial Lysosomal Fluid (ALF), a common Simulated Lungs Fluid (SLF) used in bioaccessibility assays, were selected as surrogate agents to represent the body fluids that can be contacted with coarse and fine particles, respectively. The rationale behind this choice is that only the fine fraction (PM2.5) reaches the alveoli and comes into contact with lung fluids, while the coarse fraction (2.5-10 μm), although deposited first in the pharyngeal and tracheal region, is subsequently transported by the mucociliary clearance adoral and is mainly swallowed, reaching the gastrointestinal tract, coming into contact with the gastric juice (Alpofead et al., 2016; Corona Sánchez et al., 2021; Mukhtar and Limbeck, 2013).

Secondly, the non-bioaccessible concentration was obtained after digestion of the insoluble fraction according to the European standard

method "EN-UNE 14902:2006", which consisted in an acid digestion of the filter in a microwave system (Milestone Ethos One) using closed PTFE vessels (HNO_3: H_2O_2, 4:1, up to 220 °C).

Lastly, the concentration of Mn in the leachates obtained from the bioaccessibility and total digestion tests was analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500 CE), as described in Expósito et al. (2021), where limits of detection (LOD) and quality control procedures were detailed. The following LODs were calculated for the bioaccessible and the non-bioaccessible fractions of PM10-2.5, and the bioaccessible and the non-bioaccessible fractions of PM2.5, respectively: 0.76, 2.52, 0. 59 and 0.99 ng/m^3. The percentage of samples below the LOD for the same fractions was 1.5, 40.8, 4.6 and 6.9 %, respectively.

2.4. Biomarkers sampling and analysis

Whole blood samples (7.5 mL) were obtained by venipuncture, collected in lithium heparin monovettes developed for metal determinations (Sarstedt, Nümbrecht, Germany). These samples were refrigerated for up to a maximum of 14 days until 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) at a minimum blood/alkaline solution ratio of 1/10 (w/w), and then analyzed by ICP/MS.

A washing protocol was developed for scalp hair and fingernails, based on that described by Eastman et al. (2013), which is more exhaustive than others documented, thus eliminating all exogenous metals and ensuring that only endogenous ones are quantified. This protocol consists of five stages: a first sonication for 20 min with a 0.5 % (w/v) solution of triton X-100, subsequent washing (five times) with ultrapure water, a second sonication with a 1 N HNO_3 solution, subsequent washing with the 1 N HNO_3 solution and last washing (five times) with ultrapure water, drying in the oven overnight at 65 °C. Once cleaned, samples are microwave-assisted digested (Milestone, Ethos One) at 200 °C in a solution of HNO_3/H_2O_2 at a ratio 4 /1 (v/v), and finally analyzed by ICP/MS. Details on the ICP-MS measurements of Mn concentration in all these matrices can be found in Markiv et al. (2020).

All reagents used for the treatment and preparation of the samples were of analytical grade provided by Merck and PanReac AppliChem (Darmstadt, Germany). Details on the ICP-MS measurements of Mn concentration and LODs determination in all these matrices can be found in Markiv et al. (2020). The following LODs were calculated: 0.74 μg/L for whole blood, 3.37–115.86 ng/g for scalp hair and 9.76–89.23 ng/g for fingernails. All Mn concentrations in these matrices were above the LOD.

2.5. Statistical analysis

A value of LOD/2 was assigned for Mn concentrations below the LOD. Categorical and discrete variables were expressed as counts (percentage). Continuous variables were expressed as mean and Standard Deviation (SD) and/or median and interquartile ranges (IQR). Statistical differences between groups were assessed with the Chi-square test using Yates' correction or Fisher's exact test, when appropriate, for categorical variables. Continuous variables were compared with the Student's T test (for equal or different variances, depending on the previous result in the Levene test) in relation to comparison of Means and U Mann Whitney Test in relation to comparison of Medians. The correlation between exposure and Motor test scores as continuous variables was calculated using the Spearman's rank correlation coefficient (rho).

Crude and adjusted Mean Differences (MDs) were obtained by using a linear regression model. To estimate the strength of associations, the exposure indices, biomarkers and motor tests scores were divided into dichotomous variables (low *versus* high values) according to the median and adjusted odds ratios (OR) with their 95 % confidence intervals (CI); 95 % CIs were calculated using unconditional logistic regression. The following confounders were established for inclusion in the models: age (as a continuous variable), sex, educational level (ordinal categorized), and number of years of residence (continuous). Three multivariable models were performed: the first one with age and sex, the second one adding educational level, and the last one with all covariates and also including years of residence. In addition, diet, alcohol consumption, smoking, and employment status were considered as potential confounders but did not influence model results.

The level of statistical significance was set at 0.05 and all tests were two-tailed. Data were analyzed using SPSS statistical software package 24.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Mn levels and basal characteristics of Study population

Table 1 shows the Mn levels of participants in PEMs and biomarkers as a function of proximity to IES. High SDs were observed in all of the studied fractions. Despite this variability, 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 ($\leq$ 1.5 km) from the IES compared to those residing at greater distance ($>$ 1.5 km and $\leq$10 km) considered as highly and moderately exposed respectively, and presenting a negative correlation (the shorter distance, the higher levels) (see Table S2). Regarding biomarkers, only determinations from fingernails were significantly higher among participants residing at a shorter distance ($\leq$ 1.5 km). No Mean differences were found in blood Mn levels. In relation to scalp hair Mn levels, higher values were observed for residents living at a shorter distance from the IES, but only the median comparison reached statistical significance (see Table 1). Regarding correlations, a positive non-statistically significant correlation was obtained between blood Mn levels and source distance from the IES and a negative nonsignificant correlation was obtained for scalp hair Mn levels (see Table S2).

The overall mean age of the study participants was 41.72 years [SD 13.97], with an statistically significant association between an older age and worse motor function as assessed by GP for both dom and nondom hands (see Table 2), of borderline significance for FTT ($p = 0.055$ and 0.049) for dom and nondom hands respectively (See Table S3) and also statistically significant ($p = 0.016$) for nondom hand in relation to Dynamometer assessment (See Table S4).

Ninety five out of 130 participants (73.1 %) were female. Sex (being man) was associated with statistically significant higher time to complete GP for the nondom hand (see Table 2) and being woman with lower number of taps (See Table S3) in both hands. As expected, being female was associated with statistically significant worse results for both dom and nondom hands in Dynamometer ($p < 0.001$). No male scored below 27.70 and 24.70 kg (overall median cut-off points for the dom and nondom hands) (See Table S4).

Regarding educational 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. The lower the level of education, the longer the times to complete the GP test ($p = 0.006$ and $p < 0.001$ for dom and nondom hands respectively) (see Table 2). Regarding FTT, no statistically significant differences were observed for educational level ($p = 0.161$ and $p < 0.192$ for dom and nondom hands respectively), although lower educational levels were more prevalent in those with lower number of taps (See Table S3). No significant differences were observed for the Dynamometer either (See Table S4).

The longer the number of years of residence, the worse the motor function test results with statistically significant huge differences in the GP and FTT and of smaller magnitude but also significant for the Dynamometer (see Tables 2 and S3 and S4).

In terms of employment status, some categories were associated with better or worse scores for the GP and FTT (see Tables 2 S3). After

Table 1
Mn levels in PM personal samplers (PEMs) and biomarkers as a function of proximity to Industrial Emission Source (IES).

	Source distance from IES						
	Greater distance (>1.5 km) (n = 65)		Shorter distance (≤ 1.5 km) (n = 65)		Total (n = 130)		*p value*
PEMs (ng/m^3)							
Coarse fraction (PM10-2.5)							
Bioaccessible							
Arithmetic Mean, SD	14.61	38.46	107.62	242.15	61.11	178.90	*0.003**
Geometric Mean	6.04		40.34		15.61		
Median, P95	5.62	53.99	45.91	288.27	13.61	249.11	*<0.001***
Range: min, max	0.38	300.66	2.06	1837.38	0.38	1837.38	
Interquartile Range (P25, P75)	3.26	12.75	14.61	116.42	4.5	52.19	
Non-Bioaccessible							
Arithmetic Mean, SD	4.02	7.23	19.62	51.31	11.82	37.33	*0.018*
Geometric Mean	2.27		7.36		4.09		
Median, P95	1.26	24.24	8.19	74.55	3.39	39.73	*<0.001*
Range: min, max	1.26	39.12	1.26	402.47	1.26	402.47	
Interquartile Range (P25, P75)	*1.26*	4.06	2.65	19.08	1.26	9.2	
Total (Bio + Non-Bio)							
Arithmetic Mean, SD	18.63	43.92	127.24	292.37	72.93	215.26	*0.004*
Geometric Mean	9.1		50.34		21.41		
Median, P95	7.86	73.44	53.49	344.12	16.47	289.20	*<0.001*
Range: min, max	1.64	339.79	3.32	2239.86	1.64	2239.86	
Interquartile Range (P25, P75)	*4.78*	15.02	18.43	128.18	6.79	60.83	
Fine fraction (PM2.5)							
Bioaccessible							
Arithmetic Mean, SD	23.42	40.46	109.2	178.3	66.31	135.79	*<0.001*
Geometric Mean	7.46		36.81		16.57		
Median, P95	8.07	86.23	34.23	472.05	17.05	315.47	*<0.001*
Range: min, max	0.3	245.01	1.11	899.2	0.3	899.2	
Interquartile Range (P25, P75)	2.29	29.97	11.14	148.83	4.89	55.29	
Non-Bioaccessible							
Arithmetic Mean, SD	8.32	17.27	16.96	22.85	12.64	20.64	*0.017*
Geometric Mean	4.05		8.29		5.8		
Median, P95	4.33	40.27	7.5	67.17	5.8	61.23	*<0.001*
Range: min, max	0.5	125.29	0.5	103.33	0.5	125.29	
Interquartile Range (P25, P75)	2.17	7.89	3.74	16.66	2.84	10.22	
Total (Bio + Non-Bio)							
Arithmetic Mean, SD	31.74	48.43	126.16	190.1	78.95	146.08	*<0.001*
Geometric Mean	13.96		50		26.42		
Median, P95	12.78	157.01	44.58	503.66	25	350.58	*<0.001*
Range: min, max	0.79	252.77	3.99	937.87	0.79	937.87	
Interquartile Range (P25, P75)	5.63	38.53	16.08	157.85	9.48	78.36	
All fractions (PM10)							
Total (Bio + Non-Bio)							
Arithmetic Mean, SD	50.38	81.23	253.4	440.67	151.89	331.66	*0.001*
Geometric Mean	25.4		111.33		53.18		
Median, P95	19.55	235.26	105.67	985.84	43.87	577.23	*<0.001*
Range: min, max	4.23	520.43	7.36	3145.27	4.23	3145.27	
Interquartile Range (P25, P75)	10.85	56.22	39.63	318.36	17.41	165.88	
Biomarkers							
Blood Mn (μg/L)							
Arithmetic Mean, SD	9.86	3.11	10.04	3.65	9.95	3.38	*0.759*
Geometric Mean	9.34		9.49		9.42		
Median, P95	9.68	16.63	9.16	16.13	9.58	16.01	*0.865*
Range: min, max	2.12	18.33	4.6	26.76	2.12	26.76	
Interquartile Range (P25, P75)	7.82	11.88	7.41	11.79	7.65	11.87	
Scalp hair Mn (ng/g)							
Arithmetic Mean, SD	229.88	320.02	529.64	1975.6	380.98	1422.85	*0.244*
Geometric Mean	150.06		231.76		186.82		
Median, P95	132.68	646.37	235.45	786.2	185.3	748.01	*0.005*
Range: min, max	13.22	2380.07	30.61	15730.27	13.22	15730.27	
Interquartile Range (P25, P75)	75.83	293.24	149.53	333.49	115.95	300.6	
Fingernails Mn (ng/g)							
Arithmetic Mean, SD	433.37	308.8	1440.05	1386.42	936.71	1120.51	*<0.001*
Geometric Mean	325.79		1012.3		574.28		
Median, P95	331.28	1054.05	917.87	5243.84	555.27	3549.18	*<0.001*
Range: min, max	29.29	1202.67	172.36	6437.22	29.29	6437.22	
Interquartile Range (P25, P75)	189.34	636.99	543.49	1672.19	301.7	1048.96	

SD = Standard Deviation. P25 = 25th percentile. P75 = 75th percentile. P95 = 95th percentile. * Student's T p values. ** Mann Whitney U p values.

adjusting for age, these associations disappeared (data not shown in tables). For the rest of the baseline characteristics analyzed, no statistically significant differences were observed.

3.2. Motor function results

Regarding GP, overall mean time to complete the test was 59.31 and 65.27 s (SD = 10.11 and 11.69) for dom and nondom hands, with medians of 57.77 and 61.47 respectively (see Table S5).

Table 2
Characteristics of study population as a function of Grooved Pegboard (GP) results*.

Characteristics	GP (dominant hand)							GP (nondominant hand)						
	Higher times >57.81 s (n = 65)		Lower times ≤57.80 s (n = 65)		Total (n = 130)		*p value*	Higher times >61.48 s (n = 65)		Lower times ≤61.47 s (n = 65)		Total (n = 130)		*p value*
***Age** (Mean, SD)*	49.89	13.48	33.54	8.7	41.72	13.97	*<0.001*	48.28	14.79	35.15	9.34	41.72	13.97	*<0.001*
(range: min, max)	21.00	71	20	52	20	71		20	71	21	61	20	71	
***Sex** (n, %)*							*0.075*							*0.010*
Female	43.00	66.2%	52	80.0%	95	73.1%		41	63.1%	54	83.1%	95	73.1%	
Male	22.00	33.8%	13	20.0%	35	26.9%		24	36.9%	11	16.9%	35	26.9%	
Educational level (n, %)							*0.006*							*<0.001*
Primary education	6.00	9.2%	0	0.0%	6	4.6%		6	9.2%	0	0.0%	6	4.6%	
Secondary Education/ Vocational education and Training	15.00	23.1%	7	10.8%	22	16.9%		15	23.1%	7	10.8%	22	16.9%	
High school level/ Certificate of Higher Education	16.00	24.6%	14	21.5%	30	23.1%		20	30.8%	10	15.4%	30	23.1%	
University studies (Bachelor's Degree)	10.00	15.4%	9	13.8%	19	14.6%		6	9.2%	13	20.0%	19	14.6%	
University studies (University Degree)	18.00	27.7%	35	53.8%	53	40.8%		18	27.7%	35	53.8%	53	40.8%	
***Years residing** (Mean, SD)*	19.69	14.91	10.77	10.63	15.23	13.65	*<0.001*	20.25	15.15	10.22	9.75	15.23	13.65	*<0.001*
(range: min, max)	1.00	71	1	45	1	71		1	71	1	40	1	71	
Employment status							*0.003*							*0.003*
Employed full-time	43.00	66.2%	54	83.1%	97	74.6%		40	61.5%	57	87.7%	97	74.6%	
Unemployed	3.00	4.6%	2	3.1%	5	3.8%		2	3.1%	3	4.6%	5	3.8%	
Housewife	3.00	4.6%	2	3.1%	5	3.8%		4	6.2%	1	1.5%	5	3.8%	
Retired	13.00	20.0%	0	0.0%	13	10.0%		12	18.5%	1	1.5%	13	10.0%	
Full-time student	3.00	4.6%	7	10.8%	10	7.7%		7	10.8%	3	4.6%	10	7.7%	
Smoking Status							*0.369*							*0.516*
Non-smoker	40.00	61.5%	44	67.7%	84	64.6%		39	60.0%	45	69.2%	84	64.6%	
Former	14.00	21.5%	8	12.3%	22	16.9%		13	20.0%	9	13.8%	22	16.9%	
Current	11.00	16.9%	13	20.0%	24	18.5%		13	20.0%	11	16.9%	24	18.5%	
Alcohol Status							*0.597*							*0.113*
Never	*34.00*	*52.3%*	37	56.9%	71	54.6%		31	47.7%	40	61.5%	71	54.6%	
Ever	*31.00*	*47.7%*	28	43.1%	59	45.4%		34	52.3%	25	38.5%	59	45.4%	
***Average of pure ethanol** (g/week)*							*0.104*							*0.384*
0	*34.00*	*52.3%*	37	56.9%	71	54.6%		31	47.7%	40	61.5%	71	54.6%	
1–24	*16.00*	*24.6%*	6	9.2%	22	16.9%		14	21.5%	8	12.3%	22	16.9%	
25–74	*13.00*	*20.0%*	20	30.8%	33	25.4%		18	27.7%	15	23.1%	33	25.4%	
>75	*2.00*	*3.1%*	2	3.1%	4	3.1%		2	3.1%	2	3.1%	4	3.1%	

SD = Standard Deviation.
* Higher times to complete the test indicate worse motor function.

Crude statistically significant higher times (indicating worse function) were observed when living near the IES in both hands (p = 0.024 and 0.014 respectively) (see Tables 3 and S6) with a crude MD of 3.98; 95%CI (0.52–7.43) and 5.02; 95%CI (1.04–9.00) more seconds for dom and nondom hands (see Table 3) with supporting statistically significant correlations of -0.178 and -0.258 respectively (see Table S7). After adjusting for age and sex, these MDs diminished, being of only 2.31 and 3.22 s respectively, and losing statistical significance. When educational level was added in the multivariate model, adjusted MDs of 1.22 and 2.05 s were obtained (p = 0.373 and 0.221 respectively) (see Table 3). Table S8 shows associations between source distance and GP results dichotomous categorized in higher and lower values according to median. Living near the IES was associated with a 2.92-fold increase of having GP times higher than 61.47 s for the nondom hand: Crude OR = 2.92; 95%CI (1.43–5.95), p = 0.003. Statistical significance remained after adjusting for age, sex and educational level: adjusted OR = 2.51; 95%CI (1.07–5.90), p = 0.035 (see Tables S8 and S9).

For GP and Mn levels in PEMs (in both bioaccessible and non-bioaccessible coarse&fine fractions) higher times were computed in participants with higher levels in the bivariate analysis but yielding statistical significance only for the bioaccessible-fine fraction with a crude MD of 3.56 more seconds; 95%CI (0.09–7.03), p = 0.044 (see Tables 3 and S6). This difference diminished but still yielded statistical significance after controlling for confounding (age, sex and educational level): adjusted MD = 3.01 more seconds; 95%CI (0.44–5.58), p = 0.022 (see Table 3). Positive crude and adjusted OR were obtained (indicated a higher risk of worse function) but without yielding statistical significance (see Tables S8 and S9).

In relation to biomarkers, statistically significant higher times in GP were observed in participants with higher Mn fingernails levels with a crude MD of 6.04; 95%CI (2.45–9.63) and 7.24; 95%CI (3.04–11.44) more seconds respectively for dom and nondom hands (p = 0.001) (see Tables 3 and S6) and supported by significant correlations of 0.237 and 0.275 respectively (See Table S7). After adjusting for age, sex and educational level, these associations diminished and lost statistical significance: adjusted MD for the dom hand = 1.93 more seconds; 95%CI (-0.96 to 4.82), p = 0.189 and adjusted MD for the nondom hand = 2.84 more seconds; 95%CI (-0.76 to 6.44), p = 0.121 (see Table 3). In the form of ORs, the crude associations for higher Mn fingernails levels [crude OR dom hand = 2.50; 95%CI (1.18–5.28), p = 0.017 and crude OR nondom hand = 3.11; 95%CI (1.46–6.63), p = 0.003], diminished and lost also statistical significance after adjusting for the covariates mentioned above: ORa2 dom hand = 1.36; 95%CI (0.53–3.49), p = 0.519 and ORa2 nondom hand = 2.17; 95%CI (0.89–5.29), p = 0.088 (see Tables S8 and S9).

Regarding FTT, the mean number of taps per 10 s was 68.27 and 60.25 s (SD = 11.32 and 11.72), with medians of 68.00 and 61.50 taps for the dom and nondom hands respectively (see Table S5).

No statistically significant crude or adjusted mean differences were found for exposure indices (see Table 4) but in the form of OR, statistically significant crude and adjusted OR were obtained in the nondom hand for the bioaccessible-fine fraction: ORa2 nondom hand = 2.17; 95%CI (1.03–4.57), p = 0.041 and the Total fine fraction (Bio + Non-Bio): ORa2 nondom hand = 2.15; 95%CI (1.01–4.57), p = 0.047 (see

Table 3
Mean Differences for Grooved Pegboard test (dominant and nondominant hand) according to Mn exposure indices and biomarkers.

	Grooved Pegboard (seconds)*															
	MD crude	95 %	CI	*p value*	MDa1	95 %	CI	*p value*	MDa2	95 %	CI	*p value*	MDa3	95 %	CI	*p value*
Exposure indices Source distance from IES (≤ 1.5 vs 1.5+ km)																
dom hand	3.98	0.52	7.43	*0.024*	2.31	−0.39	5.02	*0.093*	1.22	−1.48	3.92	*0.373*	0.82	−1.92	3.56	*0.554*
nondom hand	5.02	1.04	9	*0.014*	3.22	−0.05	6.5	*0.054*	2.05	−1.25	5.35	*0.221*	1.24	−2.05	4.53	*0.458*
PM personal samplers (ng/m^3)																
Coarse fraction (PM10-2.5)																
Bioaccessible (13.62+ vs ≤13.61 ng/m^3)																
dom hand	1.07	−2.44	4.59	*0.547*	0.55	−2.16	3.25	*0.691*	−0.62	−3.30	2.06	*0.647*	−1.05	−3.74	1.65	*0.443*
nondom hand	1.67	−2.4	5.73	*0.418*	1.08	−2.21	4.37	*0.517*	−0.19	−3.47	3.09	*0.910*	−1.01	−4.26	2.24	*0.54*
Non-Bioaccessible (3.39+ vs ≤3.38 ng/m^3)																
dom hand	1.21	−2.31	4.72	*0.498*	0.66	−2.04	3.36	*0.629*	−0.06	−2.68	2.57	*0.966*	−0.22	−2.83	2.39	*0.869*
nondom hand	1.2	−2.87	5.27	*0.561*	0.6	−2.68	3.89	*0.718*	−0.20	−3.42	3.01	*0.901*	−0.53	−3.67	2.62	*0.741*
Total (Bio + Non-Bio) (16.48+ vs ≤16.47 ng/m^3)																
dom hand	1.13	−2.38	4.65	*0.525*	0.48	−2.22	3.19	*0.724*	−0.67	−3.34	2.00	*0.621*	−0.99	−3.66	1.69	*0.466*
nondom hand	2.46	−1.59	6.51	*0.231*	1.76	−1.51	5.04	*0.288*	0.56	−2.72	3.83	*0.737*	−0.05	−3.27	3.18	*0.977*
Fine fraction (PM2.5)																
Bioaccessible (17.06+ vs ≤17.05 ng/m^3)																
dom hand	3.56	0.09	7.03	*0.044*	3.67	1.05	6.3	*0.006*	3.01	0.44	5.58	*0.022*	2.82	0.25	5.39	*0.032*
nondom hand	3.64	−0.38	7.67	*0.075*	3.75	0.53	6.97	*0.023*	3.00	−0.17	6.17	*0.064*	2.58	−0.54	5.7	*0.104*
Non-Bioaccessible (5.81+ vs ≤5.80 ng/m^3)																
dom hand	2.73	−0.76	6.22	*0.124*	2.75	0.09	5.4	*0.043*	1.92	−0.70	4.53	*0.150*	1.88	−0.73	4.48	*0.156*
nondom hand	1.56	−2.5	5.62	*0.449*	1.58	−1.69	4.85	*0.341*	0.60	−2.64	3.83	*0.716*	0.51	−2.64	3.67	*0.749*
Total (Bio + Non-Bio) (25.01+ vs ≤25.00 ng/m^3)																
dom hand	3.41	−0.06	6.88	*0.054*	3.21	0.57	5.85	*0.017*	2.24	−0.40	4.88	*0.096*	2.06	−0.58	4.69	*0.125*
nondom hand	3.7	−0.32	7.72	*0.071*	3.48	0.26	6.71	*0.035*	2.39	−0.86	5.63	*0.148*	2.01	−1.17	5.19	*0.213*
All fractions (PM10) (43.88+ vs ≤43.87 ng/m3)																
dom hand	1.45	−2.06	4.96	*0.416*	1.05	−1.65	3.75	*0.442*	−0.04	−2.71	2.63	*0.975*	−0.2	−2.86	2.46	*0.881*
nondom hand	2.48	−1.58	6.53	*0.229*	2.07	−1.2	5.34	*0.212*	0.90	−2.36	4.17	*0.585*	0.59	−2.6	3.79	*0.714*
Biomarkers																
Blood Mn (9.59+vs ≤9.58μg/L)																
dom hand	−5.33	−8.73	−1.94	*0.002*	−3.17	−5.87	−0.46	*0.022*	−2.68	−5.30	−0.06	*0.045*	−2.63	−5.23	−0.03	*0.048*
nondom hand	−5.24	−9.2	−1.27	*0.01*	−2.94	−6.26	0.38	*0.082*	−2.39	−5.62	0.85	*0.147*	−2.3	−5.45	0.86	*0.153*
Scalp hair Mn (185.31+vs ≤185.30 ng/g)																
dom hand	−0.28	−3.98	3.42	*0.881*	0.68	−2.22	3.57	*0.645*	0.61	−2.16	3.39	*0.663*	0.64	−2.12	3.39	*0.648*
nondom hand	−1.87	−6.14	2.41	*0.389*	−0.89	−4.43	2.66	*0.622*	−0.96	−4.37	2.45	*0.579*	−0.91	−4.24	2.42	*0.589*
Fingernails Mn (555.28+ vs ≤ 555.28 ng/g)																
dom hand	6.04	2.45	9.63	*0.001*	2.69	−0.3	5.68	*0.077*	1.93	−0.96	4.82	*0.189*	1.7	−1.17	4.58	*0.243*
nondom hand	7.24	3.04	11.44	*0.001*	3.67	−0.01	7.36	*0.051*	2.84	−0.76	6.44	*0.121*	2.37	−1.13	5.87	*0.182*

IES = Industrial Emission Source. dom = dominant. nondom = nondominant. MD = Mean Difference. MDa1 = MD adjusted for age and sex. MDa2 = MD adjusted for age, sex, and educational level. MDa3 = MD adjusted for age, sex, educational level, and years of residence. *A positive MD indicates worse motor function (higher time to complete the test) in exposed to higher Mn levels.

Table 4
Mean Differences for Finger Tapping test (dominant and nondominant hand) according to Mn exposure indices and biomarkers.

	Finger Tapping Test (number of taps in 10 s)															
	MD crude	95 %	CI	*p value*	MDa1	95 %	CI	*p value*	MDa2	95 %	CI	*p value*	MDa3	95 %	CI	*p value*
Exposure indices																
Source distance from IES (≤ 1.5 vs 1.5+ km)																
dom hand	−3.09	−7.00	0.81	*0.120*	−1.95	−5.68	1.79	*0.304*	−1.70	−5.57	2.17	*0.387*	−1.41	−5.36	2.54	*0.482*
nondom hand	−1.51	−5.58	2.57	*0.466*	−0.29	−4.20	3.63	*0.884*	0.47	−3.56	4.50	*0.819*	1.17	−2.90	5.24	*0.570*
PM personal samplers (ng/m^3)																
Coarse fraction (PM10-2.5)																
Bioaccessible (13.62+ vs ≤13.61 ng/m^3)																
dom hand	−0.26	−4.21	3.68	*0.896*	0.37	−3.34	4.09	*0.842*	0.77	−3.07	4.60	*0.693*	1.12	−2.78	5.01	*0.572*
nondom hand	−1.69	−5.77	2.38	*0.413*	−1.08	−4.96	2.79	*0.581*	0.84	−4.40	3.57	*0.838*	0.20	−3.81	4.22	*0.920*
Non-Bioaccessible (3.39+ vs ≤3.38 ng/m^3)																
dom hand	0.45	−3.50	4.39	*0.823*	0.84	−2.87	4.54	*0.656*	1.08	−2.67	4.84	*0.570*	1.22	−2.55	4.98	*0.524*
nondom hand	0.83	−3.25	4.91	*0.688*	1.23	−2.63	5.10	*0.530*	1.72	−2.18	5.61	*0.384*	1.98	−1.89	5.84	*0.314*
Total (Bio + Non-Bio) (16.48+ vs ≤16.47 ng/m^3)																
dom hand	−0.72	−4.67	3.22	*0.717*	−0.29	−4.00	3.42	*0.877*	0.05	−3.78	3.88	*0.978*	0.30	−3.57	4.17	*0.878*
nondom hand	−1.54	−5.62	2.54	*0.457*	−1.10	−4.96	2.77	*0.576*	−0.43	−4.41	3.54	*0.830*	0.04	−3.95	4.02	*0.986*
Fine fraction (PM2.5)																
Bioaccessible (17.06+ vs ≤17.05 ng/m^3)																
dom hand	−0.79	−4.73	3.16	*0.694*	−0.45	−4.16	3.25	*0.809*	−0.23	−3.99	3.53	*0.903*	−0.05	−3.84	3.73	*0.978*
nondom hand	−2.99	−7.04	1.07	*0.147*	−2.71	−6.55	1.14	*0.166*	−2.31	−6.19	1.58	*0.242*	−1.98	−5.86	1.90	*0.314*
Non-Bioaccessible (5.81+ vs ≤5.80 ng/m^3)																
dom hand	0.17	−3.78	4.11	*0.932*	0.04	−3.67	3.74	*0.985*	0.33	−3.46	4.11	*0.865*	0.36	−3.43	4.15	*0.851*
nondom hand	0.89	−3.19	4.97	*0.666*	0.77	−3.09	4.64	*0.693*	1.39	−2.53	5.31	*0.485*	1.45	−2.44	5.34	*0.461*
Total (Bio + Non-Bio) (25.01+ vs ≤25.00 ng/m^3)																
dom hand	−0.29	−4.24	3.65	*0.884*	−0.07	−3.77	3.63	*0.969*	0.29	−3.54	4.11	*0.883*	0.44	−3.40	4.29	*0.819*
nondom hand	−2.12	−6.19	1.95	*0.304*	−1.91	−5.76	1.94	*0.328*	−1.30	−5.26	2.66	*0.517*	−1.01	−4.96	2.95	*0.615*
All fractions (PM10) (43.88+ vs ≤43.87 ng/m3)																
dom hand	−0.05	−3.99	3.90	*0.982*	−0.24	−3.95	3.47	*0.898*	0.10	−3.72	3.93	*0.959*	0.23	−3.61	4.06	*0.907*
nondom hand	−1.60	−5.68	2.48	*0.439*	−1.74	−5.60	2.12	*0.374*	−1.13	−5.09	2.84	*0.574*	−0.89	−4.84	3.06	*0.656*
Biomarkers																
Blood Mn (9.59+ vs ≤9.58μg/L)																
dom hand	1.37	−2.57	5.31	*0.493*	1.49	−2.28	5.27	*0.435*	1.35	−2.46	5.16	*0.484*	1.31	−2.50	5.13	*0.497*
nondom hand	2.52	−1.54	6.58	*0.221*	2.47	−1.46	6.40	*0.216*	2.17	−1.77	6.11	*0.278*	2.10	−1.82	6.01	*0.291*
Scalp hair Mn (185.31+ vs ≤185.30 ng/g)																
dom hand	4.36	0.40	8.33	*0.031*	2.62	−1.24	6.47	*0.182*	2.64	−1.22	6.50	*0.178*	2.62	−1.24	6.48	*0.181*
nondom hand	3.57	−0.57	7.71	*0.091*	1.75	−2.24	5.74	*0.386*	1.79	−2.17	5.76	*0.372*	1.75	−2.17	5.67	*0.378*
Fingernails Mn (555.28+ vs ≤ 555.28 ng/g)																
dom hand	−3.74	−8.01	0.52	*0.085*	−1.92	−6.02	2.18	*0.355*	−1.78	−5.94	2.39	*0.399*	−1.72	−5.92	2.48	*0.419*
nondom hand	−5.90	−10.14	−1.65	*0.007*	−4.27	−8.44	−0.10	*0.045*	−3.93	−8.15	0.29	*0.067*	−3.69	−7.91	0.54	*0.087*

IES = Industrial Emission Source. dom = dominant. nondom = nondominant. MD = Mean Difference. MDa1 = MD adjusted for age and sex. MDa2 = MD adjusted for age, sex, and educational level. MDa3 = MD adjusted for age, sex, educational level, and years of residence. *A negative MD indicates worse motor function (lower number of taps in 10 s) in exposed to higher Mn levels.

Tables S11 and S12).

In relation to biomarkers, a crude lower number of taps in the FTT were observed in participants with higher Mn fingernails levels: crude MD for the dom hand = -3.74 (3.74 lower taps); 95%CI (8.01 to 0.53), p = 0.085 and crude MD for the nondom hand = -5.90; 95%CI (-10.14 to -1.65), p = 0.007 (see Tables 4 and S10). After adjusting for the age, sex and educational level, these associations also diminished, losing statistical significance: adjusted MD for the dom hand = -1.78; 95%CI (-5.94 to 2.39), p = 0.399 and adjusted MD for the nondom = -3.93; 95%CI (-8.15 to 0.29), p = 0.067 (see Table 4). In the form of ORs, crude and adjusted higher associations were observed also for the nondom hand, but without yielding statistical significance: ORa2 nondom hand = 1.58; 95%CI (0.70–3.57), p = 0.266 (see Tables S11 and S12).

Regarding Dynamometer, the mean of the grip strength was 29.35 and 26.91 kg (SD = 9.30 and 9.16), with medians of 27.70 y 24.70 kg for the dom and nondom hands respectively. The medians when stratified

Table 5
Mean Differences for Dynamometer test (dominant and nondominant hand) according to Mn exposure indices and biomarkers.

	Dynamometer (kg)															
	MD crude	95 %	CI	*p value*	MDa1	95 %	CI	*p value*	MDa2	095	CI	*p value*	MDa3	95 %	CI	*p value*
Exposure indices																
Source distance from IES (≤ 1.5 vs 1.5+ km)																
dom hand	−1.49	−4.72	1.74	*0.363*	−0.02	−1.87	1.83	*0.984*	−0.32	−2.23	1.59	0.741	−0.13	−2.08	1.81	*0.892*
nondom hand	−0.75	−3.94	2.44	*0.642*	0.67	−1.34	2.68	*0.511*	0.12	−1.93	2.17	*0.907*	0.42	−1.66	2.50	*0.691*
PM personal samplers (ng/m^3)																
Coarse fraction (PM10-2.5)																
Bioaccessible (13.62+ vs ≤13.61 ng/m^3)																
dom hand	−0.06	−3.30	3.19	*0.973*	0.98	−0.85	2.80	*0.291*	0.75	−1.13	2.64	0.429	0.96	−0.95	2.87	*0.322*
nondom hand	0.67	−2.52	3.86	*0.677*	1.65	−0.32	3.62	*0.100*	1.19	−0.83	3.20	*0.245*	1.50	−0.54	3.53	*0.148*
Non-Bioaccessible (3.39+ vs ≤3.38 ng/m^3)																
dom hand	−0.67	−3.90	2.58	*0.685*	−0.18	−2.01	1.65	*0.844*	−0.36	−2.21	1.49	*0.698*	−0.29	−2.15	1.56	*0.755*
nondom hand	−0.65	−3.84	2.54	*0.688*	−0.19	−2.18	1.80	*0.850*	−0.54	−2.53	1.44	0.588	−0.44	−2.42	1.54	*0.661*
Total (Bio + Non-Bio) (16.48+ vs ≤16.47 ng/m^3)																
dom hand	0.52	−2.72	3.76	*0.750*	1.05	−0.78	2.87	*0.258*	0.83	−1.05	2.71	*0.384*	0.98	−0.91	2.88	*0.307*
nondom hand	1.34	−1.85	4.52	*0.408*	1.84	−0.13	3.80	*0.066*	1.39	−0.62	3.40	*0.172*	1.62	−0.39	3.63	*0.113*
Fine fraction (PM2.5)																
Bioaccessible (17.06+ vs ≤17.05 ng/m^3)																
dom hand	0.27	−2.98	3.51	*0.872*	1.06	−0.76	2.88	*0.253*	0.90	−0.94	2.75	*0.334*	1.02	−0.84	2.87	*0.280*
nondom hand	0.35	−2.84	3.55	*0.827*	1.09	−0.89	3.07	*0.277*	0.76	−1.22	2.74	*0.448*	0.92	−1.06	2.90	*0.360*
Non-Bioaccessible (5.81+ vs ≤5.80 ng/m^3)																
dom hand	−0.77	−4.01	2.47	*0.639*	−1.05	−2.87	0.77	*0.255*	−1.32	−3.17	0.53	*0.160*	−1.30	−3.15	0.55	*0.166*
nondom hand	0.64	−2.55	3.83	*0.692*	0.38	−1.61	2.36	*0.707*	−0.05	−2.05	1.94	*0.957*	−0.03	−2.02	1.96	*0.979*
Total (Bio + Non-Bio) (25.01+ vs ≤25.00 ng/m^3)																
dom hand	0.91	−2.33	4.14	*0.581*	1.26	−0.56	3.07	*0.173*	1.05	−0.82	2.92	0.268	1.15	−0.73	3.03	*0.229*
nondom hand	1.33	−1.85	4.51	*0.410*	1.66	−0.31	3.63	*0.097*	1.21	−0.80	3.21	0.237	1.34	−0.66	3.35	*0.187*
All fractions (PM10) (43.88+ vs ≤43.87 ng/m3)																
dom hand	2.70	−0.51	5.91	*0.098*	2.03	0.24	3.82	*0.027*	1.88	0.02	3.73	*0.047*	1.96	0.10	3.81	*0.039*
nondom hand	3.37	0.24	6.51	*0.035*	2.75	0.83	4.68	*0.005*	2.37	0.40	4.35	*0.019*	2.49	0.52	4.46	*0.014*
Biomarkers																
Blood Mn (9.59+ vs ≤9.58μg/L)																
dom hand	−1.19	−4.43	2.04	*0.467*	0.45	−1.42	2.31	*0.637*	0.58	−1.30	2.45	*0.542*	0.56	−1.32	2.43	*0.557*
nondom hand	−2.08	−5.25	1.09	*0.197*	−0.63	−2.66	1.40	*0.540*	−0.39	−2.40	1.63	*0.705*	−0.42	−2.42	1.59	*0.681*
Scalp hair Mn (185.31+ vs ≤185.30 ng/g)																
dom hand	4.00	0.77	7.23	*0.016*	0.54	−1.33	2.41	*0.569*	0.52	−1.34	2.38	*0.580*	0.51	−1.35	2.36	*0.589*
nondom hand	3.42	0.23	6.62	*0.036*	0.22	−1.89	2.32	*0.839*	0.19	−1.89	2.26	0.858	0.17	−1.89	2.24	*0.870*
Fingernails Mn (555.28+ vs ≤ 555.28 ng/g)																
dom hand	−1.83	−5.31	1.65	*0.299*	−0.15	−2.14	1.84	*0.883*	−0.25	−2.26	1.77	*0.809*	−0.22	−2.25	1.82	*0.834*
nondom hand	−1.17	−4.55	2.21	*0.495*	0.54	−1.52	2.60	*0.606*	0.32	−1.76	2.39	0.763	0.39	−1.70	2.48	*0.712*

IES = Industrial Emission Source. dom = dominant. nondom = nondominant. MD = Mean Difference. MDa1 = MD adjusted for age and sex. MDa2 = MD adjusted for age, sex, and educational level. MDa3 = MD adjusted for age, sex, educational level, and years of residence*A negative MD indicates worse motor function (lower grip strength) in exposed to higher Mn levels.

by sex were 40.10 kg for the dom hand in males and 24.80 kg in females; and 38.80 kg for the nondom hand in males and 23.30 kg in females (see Table S5).

Mixed crude MDs were obtained (from the 22 crude test, 10 were negative indicating a worse grip strength in exposed, and 12 were positive. After adjusting for age, sex and educational level, the positive MDs remained positive (two of them yielding statistical significance). None of the adjusted negative MDs reached statistical significance (see Tables 5 and S13). When Dynamometer results were dichotomous categorized into high *vs* low values with different cut-off points for women and men, all OR were not statistically significant, but most of them were <1 against the hypothesis of a worse grip strength among exposed (see Tables S14 and S15).

For Mn-Blood levels, lower times to complete the test were observed (contrary to our hypothesis) in relation to GP (especially for dom hand with significant adjusted MD results) (see Table 3). Nonsignificant lower times were obtained in relation to FTT in both dom and nondom hands (contrary also to our hypothesis) (see Table 4). Regarding Dynamometer, mixed results were obtained (see Table 5). For scalp hair, mixed results were obtained for GP whereas nonsignificant results contrary to our hypothesis were obtained for FTT and Dynamometer, supported by correlation results (See Tables 3–5 and S7).

4. Discussion

Each of our motor test analyzed evaluates a different domain. GP test evaluates "eye-hand coordination and motor speed", FTT evaluates "self-directed manual speed" and Dynamometer evaluates "grip strength" (Ruiz-Azcona et al., 2021; Strauss et al., 2006).

Among the three motor tests analyzed, our study supports poorer motor function, especially as assessed by GP test, particularly in terms of "proximity to IES", "bioaccessible-fine fraction as determined by PEMs", and "Mn fingernails levels". However, our findings were clearly affected by confounding, and only the adjusted MD for Mn bioaccessible-fine fraction remained of sufficient magnitude to maintain statistical significance. The rest associations diminished, and although they remained indicating a worse motor function, they lost statistical significance. It possibly denotes that the statistical power of our study to detect small adjusted differences was not high enough with our final sample size of 130 participants, suggesting that, statistical power was not enough to achieve a low standard error to rule out the role of chance in our results. In any case, our results support the GP results of the studies published by Bowler et al. (2012) and Viana et al. (2014), allowing to obtain a more precise estimation of the effect size in further meta-analyses.

Regarding FTT, associations in relation to proximity to IES, PEMs determinations and fingernails were also affected by confounding, but would support also the Bowler et al. (2012) and Lucchini et al. (2014) results of this test indicating a worse motor function.

To the best of our knowledge, results on Mn exposure and "hand strength" as determined by Dynamometer have only been published in one study (Bowler et al., 2012) without observing an association. As expected, large differences have been found in our study according to sex. After adjusting for it, most of our MDs association were negative (supporting a negative association) but without reaching statistical significance. When results were dichotomous categorized into high *vs* low values, most of ORs were <1 against the hypothesis of a worse grip strength, so very inconclusive and mixed results were obtained.

In relation to "years of residence" as a confounding variable, on the one hand, the variable years of residence was associated in our study with motor function, meeting the first criterion to consider a variable as a confounding variable (Rothman, 1986). On the other hand, this variable can be interpreted as part of the exposure itself instead as a confounding variable, with exposure being understood as a composite resulting from the product of intensity and duration. In addition, a correlation between age and years residing could exist. Therefore, two multivariate models were performed, the first one with all predefined covariates but excluding this variable and the second one including it. As expected, the latter multivariate model provided the lowest MD and OR values, reflecting the adjusted effect of exposure intensity by itself, with independence of years of exposure. Apart from confounding, the presence of other potential biases have been trying to be minimized by the use of the same schedules and procedures in all participants, including the order of test by a single trained investigator under the same conditions, to avoid any differential misclassification. Lastly, as this is an exploratory study, another limitation relates to the high number of comparisons made in our study; and thus, we cannot rule out that some of the associations were spurious or due to chance (*i.e.*, false-positive associations) by a multiple-test concern specially as refers to our multiple coarse & fine and bioaccessible & non-bioaccessible fractions results. Nevertheless, the concordance and homogeneity between results among the different analytical strategies in the form of Spearman correlations, MDs and ORs would support the internal validity of results.

Our study, as the rest of published studies on the relationship between environmental Mn airborne exposure in adults and motor function, has a cross-sectional design, with the limitations inherent to this design including the lack of longitudinal data on both the Mn exposure and the motor function determinations. With regards to exposure, most of our participants met both residency criteria suggesting a continuous and stable long-term exposure that covering also the potential latency period between exposure and the onset of effects on motor function. In any case, further follow-up studies are clearly needed to deep in the motor function effects derived from the environmental airborne Mn exposure. The clinical relevance of possible poorer motor function results of small magnitude in addition to the influence of aging must also be elucidated in order to obtain scientific evidence of the long term public health Mn exposure consequences for all ages.

While there are no longitudinal studies in the specific Mn environmental field in adults in relation to motor function, some cohort studies have been conducted in the occupational field. Mn alloy workers were examined in a follow-up study 14 years after exposure ceased (1990–2004 at a Canadian facility), having poorer scores compared to referents both in the initial and follow-up examinations for several motor tasks of the Luria Motor Scale (Bouchard et al., 2007). After 3.5 years of cessation of confined space welding, Bowler et al. (2011) reported that motor dexterity/tactile function and graphomotor tremor improved significantly, while psychomotor speed remained unchanged. Unified Parkinson Disease Rating Scale motor subsection (UPDRS) results did not change compared to baseline, whereas rigidity, dominant postural hand tremor and body sway worsened. Racette et al. (2017) study supported a Mn dose-dependent progression of parkinsonism in Mn exposed welders as assessed by the UPDRS part 3. In contrast, a nationwide record linkage study in Sweden did not offer support for a relation between being employment as a welder and Parkinson's disease or any other specific basal ganglia and movement disorders (Fored et al., 2006). Evidence of association was not found either in a large Danish cohort study (Kenborg et al., 2012). In Washington, US, 56 asymptomatic welder trainees exposed to low levels of Mn with no previous occupational Mn exposure were observed over the course of a five-quarter traineeship. When adjusting for possible learning effects, there were no associations between cumulative exposure and UPDRS part 3 score or Grooved Pegboard time. Interestingly, in a subset of 17 welders in which T1-weighted magnetic resonance images were performed, the basal ganglia exhibited statistically significant increases in signal intensity in relation to increased cumulative Mn exposure, suggesting that T1-weighted changes can be detected in the brain even at very low levels of exposure among humans before any clinically evident deficits, suggesting the possible identification of a T1 threshold of toxicity at which clinical symptoms begin to manifest in a continued follow-up (Baker et al., 2015). Lastly, an exploratory cohort study from southern Sweden, evaluated associations between welding and expression of 87 putative neurology-related proteins in serum, identifying five of them that were differentially expressed (Gliga et al., 2020). An

increasing number of longitudinal studies is also available evaluating Mn exposure during pregnancy, childhood and at youth ages and its adverse effects at different ages including motor function. Adverse motor function associations have been reported in Italian adolescents 11–14 years old (Lucchini et al., 2012), and at 2 years of age in relation to in utero exposure (Lin et al., 2013), but no associations have been reported in others (Gunier et al., 2015; Claus Henn et al., 2017; Andiarena et al., 2017; Soler-Blasco et al., 2020).

In a general context, the potential health effects from the metal(loid)s present in PM depend upon the solubility of elements in the human body. This solubility is influenced by the chemical speciation of these metal(loid)s and by the size and shape of particles (Kelly and Fussell, 2012), but also by the chemical composition of the leaching fluid (*i.e.*, the biological fluid where PM will be partially dissolved). In this context, bioaccessibility is defined as the fraction of the total metal(loid) concentration released from an environmental matrix into a synthetic biological fluid and becomes available for absorption (Manjón et al., 2020). Whereas conventional studies only consider the total concentration of some trace metals in PM, the bioaccessible concentration of PM-bound trace metal(loid)s instead of total content may better represent the exposure risk of such pollutants in humans (Hernández-Pellón et al., 2018; Weggeberg et al., 2019). Our results in GP and FTT for the fine bioaccessible fraction would be supported by this rational. Nevertheless, no great differences were found when comparing bio *versus* non-bioaccessible in the coarse fraction, supporting the hypothesis that the fine mode can induce worse motor function than the coarse one.

Human biomonitoring, is defined as the measurement or estimation of human exposure to environmental contaminants by determining the concentration of contaminants and/or their metabolites or other biological parameters in biological samples (blood, hair, nails, *etc.*) (Democophes, 2012). The usefulness of a biomarker should be evaluated by its ability to predict health effects; it should also be able to anticipate any deterioration as a result of short- or long-term exposure (Viana et al., 2014; Zheng et al., 2011). Currently there is no consensus on which biomarker better defines the dose-effect relationship for the specific case of Mn exposure (Fernández-Olmo et al., 2020).

Blood aims to estimate short term exposure (Aschner et al., 2007; Michalke and Fernsebner, 2014; Ntihabose et al., 2018). The hair growth rate of approximately 1 cm per month provides an exposure estimate of 1–6 months (Haynes et al., 2015) whereas the slower growth rate of nails allows to estimate low-level chronic exposure (Viana et al., 2014). Some authors state that blood is not a good biomarker of Mn exposure because the average time it remains in blood is much shorter than in tissues and intracellular compartments (Zheng et al., 2011). This seems to be particularly true when Mn is inhaled, so in occupational context, although higher levels of Mn in blood of individuals exposed to high concentrations of Mn in workplaces are found, it is difficult to quantify how the pulmonary uptake of Mn-bearing particles contributes to the overall increase of Mn in blood (Roth, 2006). Our results contrary to our hypothesis for Blood Mn, and our positive correlation between source distance from the IES and Blood Mn levels (contrary also to our hypothesis) should be considered in this context (estimation of short-term environmental exposure in one hand and no specific appropriateness for inhaled Mn exposure in the other hand). In addition, Mn is an essential micronutrient so its concentration is metabolically regulated in the blood supply. In this regard, other authors consider that hair and nails are better Mn specific biomarkers for epidemiological purposes because of their long-term exposure, although the mechanisms of Mn uptake in hair are not well known either (Bouchard et al., 2011; Coetzee et al., 2016; Haynes et al., 2015; Viana et al., 2014). Our homogeneous results for fingernails in relation to motor functions test results and our negative significant correlation with source distance from the IES, support the consideration of nails as a promising biomarker, in agreement with the recent literature for both fingernails (Butler et al., 2019; Lucas et al., 2015; Viana et al., 2014) and toenails (Ntihabose et al., 2018; Reis et al., 2015; Rodrigues et al., 2018a, b).

Lastly, our study shows variability with relatively high SDs of Mn concentrations in all the studied fractions; such variations in airborne Mn concentrations measured in the personal samplers are likely due to differences in emissions, weather, including seasonal changes, and the amount of time people spent outdoors. In spite of this variability, a correlation in adults between source distance from the IES and Mn airborne levels was observed, also maintained when differentiating between coarse & fine and bioaccessible & non-bioaccessible fractions, reflecting that source distance from the IES is a valid surrogate of overall Mn airborne exposure, and contextualizing our study population as exposed to high levels of airborne Mn when comparing with reference guideline given by WHO (150 ng/m^3) and the Reference Concentration (RfC) given by US EPA (50 ng/m^3) (annual basis). They are also contextualized as highly exposed when compared to published studies as ours, which have been conducted in areas near Mn ferroalloys plants: In Valcamonica (Italy), means of 26.4 ng/m^3 using PM10 PEMs have been reported (Lucchini et al., 2014) and mean modelled Mn values of 180 ng/m^3 in PM10 and 50 ng/m^3 in PM2.5, were reported in Marietta (US) (Bowler et al., 2012, 2016; Kim et al., 2011). Lastly, a mean of 151 ng/m^3 in PM2.5 has been reported in Simões Filho (Brazil) (Menezes-Filho et al., 2009), which is the same area studied by Viana et al. (Viana et al., 2014), where a Mn ferroalloys plant is located.

As a conclusion, our statistically significant adjusted results for the Mn bioaccessible-fine fraction and the GP test results, together with the available evidence, support an association between chronic Mn exposure and effects on motor function, and the need to regulate the airborne Mn levels.

Funding

This work was supported by the Spanish Ministry of Science, Innovation and Universities through the CTM2017-82636-R Project. This funding source was not involved in the study design; data collection, analysis, or interpretation; the writing of the article; or the decision to submit for publication. Bohdana Markiv also thanks this Ministry for the PhD grant awarded, PRE2018-085152.

Conflict of interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Laura Ruiz-Azcona: Investigation, Writing - original draft, Formal analysis. **Bohdana Markiv:** Investigation, Resources. **Andrea Expósito:** Investigation, Resources. **Isabel González-Aramburu:** Investigation, Resources. **María Sierra:** Investigation, Resources. **Ignacio Fernández-Olmo:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition. **Miguel Santibáñez:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition.

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.

Acknowledgments

We would like to thank Ana Pozueta Cantudo and María García Martínez, from the neurology service of the Hospital Universitario Marqués de Valdecilla, and Paula Parás Bravo, Maria Paz Zulueta and Carmen Sarabia Cobo at the University of Cantabria, for their professional support in the design and implementation of the study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.neuro.2021.10.005.

References

Alpofead, J.A.H., Davidson, C.M., Littlejohn, D., 2016. Oral bioaccessibility tests to measure potentially toxic elements in inhalable particulate matter collected during routine air quality monitoring. Anal. Methods 8, 5466–5474. https://doi.org/10.1039/c6ay01403h.

Andiarena, A., Balluerka, N., Gorostiaga, A., Ibarluzea, J., 2017. Neuropsychological assessment at preschool age: adaptation and validation of the McCarthy scales of children's abilities to 4 year-old basque-speaking children. Span. J. Psychol. 23 (20), E49. https://doi.org/10.1017/sjp.2017.49.

Arruti, A., Fernández-Olmo, I., lrabien, A., 2010. Evaluation of the contribution of local sources to trace metals levels in urban PM2.5 and PM10 in the Cantabria region (Northern Spain). J. Environ. Monit. 12 (7), 1451–1458. https://doi.org/10.1039/b926740a.

Arruti, A., Fernández-Olmo, I., lrabien, A., 2011a. Regional evaluation of particulate matter composition in an Atlantic coastal area (Cantabria region, northern Spain): spatial variations in different urban and rural environments. Atmos. Res. 101 (1), 280–293. https://doi.org/10.1016/j.atmosres.2011.03.001.

Arruti, A., Fernández-Olmo, I., lrabien, A., 2011b. lmpact of the global economic crisis on metal levels in particulate matter (PM) at an urban area in the Cantabria Region (Northern Spain). Environ. Pollut. 159 (5), 1129–1135. https://doi.org/10.1016/j.envpol.2011.02.008.

Aschner, M., Guilarte, T.R., Schneider, J.S., Zheng, W., 2007. Manganese: recent advances in understanding its transport and neurotoxicity. Toxicol. Appl. Pharmacol. 221, 131–147. https://doi.org/10.1016/j.taap.2007.03.001.

Baker, M.G., Criswell, S.R., Racette, B.A., Simpson, C.D., Sheppard, L., Checkoway, H., Seixas, N.S., 2015. Neurological outcomes associated with low-level manganese exposure in an inception cohort of asymptomatic welding trainees. Scand. J. Work Environ. Health 41 (1), 94–101. https://doi.org/10.5271/sjweh.3466.

Bornstein, R.A., 1986. Normative data on intermanual differences on three test of motor performance. J. Clin. Exp. Neuropsychol. 8, 12–20. https://doi.org/10.1016/j.taap.2007.03.001.

Bouchard, M., Mergler, D., Baldwin, M., Panisset, M., Bowler, R., Roels, H.A., 2007. Neurobehavioral functioning after cessation of manganese exposure: a follow-up after 14 years. Am. J. Ind. Med. 50 (11), 831–840. https://doi.org/10.1002/ajim.20407.

Bouchard, M.F., Sauvé, S., Barbeau, B., Legrand, M., Brodeur, M.È., Bouffard, T., Limoges, E., Bellinger, D.C., Mergler, D., 2011. Intellectual impairment in school-age children exposed to manganese from drinking water. Environ. Health Perspect. 119, 138–143. https://doi.org/10.1289/ehp.1002321.

Bowler, R.M., Gysens, E., Diamond, S., Nakagawa, M., Drezgic, M., Roels, H.A., 2006a. Manganese exposure: neuropsychological and neurological symptoms and effects in welders. Neurotoxicology 27, 315–326. https://doi.org/10.1289/ehp.1002321.

Bowler, R.M., Koller, W., Schulz, P.E., 2006b. Parkinsonism due to manganism in a welder: neurological and neuropsychological sequelae. Neurotoxicology 27, 237–332. https://doi.org/10.1016/j.neuro.2005.10.011.

Bowler, R.M., Gocheva, V., Harris, M., Ngo, L., Abdelouahab, N., Wilkinson, J., Doty, R. L., Park, R., Roels, H.A., 2011. Prospective study on neurotoxic effects in manganese-exposed bridge construction welders. Neurotoxicology 32 (5), 596–605. https://doi.org/10.1016/j.neuro.2011.06.004.

Bowler, R.M., Harris, M., Gocheva, V., Wilson, K., Kim, Y., Davis, S.I., Bollweg, G., Lobdell, D.T., Ngo, L., Roels, H.A., 2012. Anxiety affecting parkinsonian outcome and motor efficiency in adults of an Ohio community with environmental airborne manganese exposure. Int. J. Hyg. Environ. Health 215, 393–405. https://doi.org/10.1016/j.ijheh.2011.10.005.

Bowler, R.M., Kornblith, E.S., Gocheva, V.V., Colledge, M.A., Bollweg, G., Kim, Y., Beseler, C.L., Wright, C.W., Adams, S.W., Lobdell, D.T., 2015. Environmental exposure to manganese in air: associations with cognitive functions. Neurotoxicology 49, 139–148. https://doi.org/10.1016/j.neuro.2015.06.004.

Bowler, R.M., Beseler, C.L., Gocheva, V.V., Colledge, M., Kornblith, E.S., Julian, J.R., Kim, Y., Bollweg, G., Lobdell, D.T., 2016. Environmental exposure to manganese in air: associations with tremor and motor function. Sci. Total Environ. 541, 646–654. https://doi.org/10.1016/j.scitotenv.2015.09.084.

Butler, L., Gennings, C., Peli, M., Borgese, L., Placidi, D., Zimmerman, N., Hsu, H.H.L., Coull, B.A., Wright, R.O., Smith, D.R., Lucchini, R.G., Claus Henn, B., 2019. Assessing the contributions of metals in environmental media to exposure biomarkers in a region of ferroalloy industry. J. Expo. Sci. Environ. Epidemiol. 29, 674–687. https://doi.org/10.1038/s41370-018-0081-6.

Chen, P., Bornhorst, J., Aschner, M., 2018. Manganese metabolism in humans. Front. Biosci. 23, 155–1679. https://doi.org/10.2741/4665.

Claus Henn, B., Bellinger, D.C., Hopkins, M.R., Coull, B.A., Ettinger, A.S., Jim, R., Hatley, E., Christiani, D.C., Wright, R.O., 2017. Maternal and cord blood manganese concentrations and early childhood neurodevelopment among residents near a mining-impacted superfund site. Environ. Health Perspect. 125 (6), 067020 https://doi.org/10.1289/EHP925.

Coetzee, D.J., McGovern, P.M., Rao, R., Harnack, L.J., Georgieff, M.K., Stepanov, I., 2016. Measuring the impact of manganese exposure on children's neurodevelopment: advances and research gaps in biomarker-based approaches. Environ. Health 15, 1–20. https://doi.org/10.1186/s12940-016-0174-4.

Corona Sánchez, J.E., del C.A. González Chávez, M., Carrillo González, R., Scheckel, K., Tapia Maruri, D., García Cue, J.L., 2021. Metal(loid) bioaccessibility of atmospheric particulate matter from mine tailings at Zimapan, Mexico. Environ. Sci. Pollut. Res. 28, 19458–19472. https://doi.org/10.1007/s11356-020-11887-6.

Democophes, 2012. . Demonstration of a Study to Coordinate and Perform Human Biomonitoring on a European Scale. Available in: http://democophes.blogs.isciii.es/.

Denys, S., Caboche, J., Tack, K., Rychen, G., Wragg, J., Cave, M., Jondreville, C., Feidt, C., 2012. In vivo validation of the unified BARGE method to assess the bioaccessibility of arsenic, antimony, cadmium, and lead in soils. Environ. Sci. Technol. 46, 6252–6260. https://doi.org/10.1021/es3006942.

Dlamini, W.W., Nelson, G., Nielsen, S.S., Racette, B.A., 2020. Manganese exposure, parkinsonian signs, and quality of life in South African mine workers. Am. J. Ind. Med. 63 (1), 36–43. https://doi.org/10.1002/ajim.23060.

Eastman, R.R., Jursa, T.P., Benedetti, C., Lucchini, R., Smith, D.R., 2013. Hair as a biomarker of environmental manganese exposure. Environ. Sci. Technol. 47, 1629–1637. https://doi.org/10.1021/es3035297.

European Pharmacopoeia, 2010. Recommendations on methods for dosage forms testing. Eur. Pharmacopoeia 8.0 727–729.

Expósito, A., Markiv, B., Ruiz-Azcona, L., Santibáñez, M., Fernández-Olmo, I., 2021. Understanding how methodological aspects affect the release of trace metal(loid)s from urban dust in inhalation bioaccessibility tests. Chemosphere 267, 1–10. https://doi.org/10.1016/j.chemosphere.2020.129181.

Fernández-Olmo, I., Mantecón, P., Markiv, B., Ruiz-Azcona, L., Santibáñez, M., 2020. A review on the environmental exposure to airborne manganese, biomonitoring, and neurological/neuropsychological outcomes. Rev. Environ. Contam. Toxicol. https://doi.org/10.1007/398_2020_46.

Fored, C.M., Fryzek, J.P., Brandt, L., Nise, G., Sjögren, B., McLaughlin, J.K., Blot, W.J., Ekbom, A., 2006. Parkinson's disease and other basal ganglia or movement disorders in a large nationwide cohort of Swedish welders. Occup. Environ. Med. 63 (2), 135–140. https://doi.org/10.1136/oem.2005.022921.

Fulk, F., Haynes, E.N., Hilbert, T.J., Brown, D., Petersen, D., Reponen, T., 2016. Comparison of stationary and personal air sampling with an air dispersion model for children's ambient exposure to manganese. J. Exposure Sci. Environ. Epidemiol. 26, 494–502. https://doi.org/10.1038/jes.2016.30.

Gliga, A.R., Taj, T., Wahlberg, K., Lundh, T., Assarsson, E., Hedmer, M., Albin, M., Broberg, K., 2020. Exposure to mild steel welding and changes in serum proteins with putative neurological function-a longitudinal study. Front. Public Health 8, 422. https://doi.org/10.3389/fpubh.2020.00422.

González-Antuña, A., Camacho, M., Henríquez-Hernández, L.A., Boada, L.D., Almeida-González, M., Zumbado, M., Luzardo, O.P., 2017. Simultaneous quantification of 49 elements associated to e-waste in human blood by ICP-MS for routine analysis. MethodsX 4, 328–334. https://doi.org/10.1016/j.mex.2017.10.001.

Graney, J.R., Landis, M.S., Norris, G.A., 2004. Concentrations and solubility of metals from indoor and personal exposure PM 2.5 samples. Atmos. Environ. 38, 237–247. https://doi.org/10.1016/j.atmosenv.2003.09.052.

Guarneros, M., Ortiz-Romo, N., Alcaraz-Zubeldia, M., Drucker-Colín, R., Hudson, R., 2013. Nonoccupational environmental exposure to manganese is linked to deficits in peripheral and central olfactory function. Chem. Senses 38, 783–791. https://doi.org/10.1093/chemse/bjt045.

Gunier, R.B., Arora, M., Jerrett, M., Bradman, A., Harley, K.G., Mora, A.M., Kogut, K., Hubbard, A., Austin, C., Holland, N., Eskenazi, B., 2015. Manganese in teeth and neurodevelopment in young Mexican-American children. Environ. Res. 142, 688–695. https://doi.org/10.1016/j.envres.2015.09.003. PMID: 26381693; PMCID: PMC4696558.

Haynes, E.N., Ryan, P., Chen, A., Brown, D., Roda, S., Kuhnell, P., Wittberg, D., Terrell, M., Reponen, T., 2012. Assessment of personal exposure to manganese in children living near a ferromanganese refinery. Sci. Total Environ. 427-42, 19–25. https://doi.org/10.1016/j.scitotenv.2012.03.037.

Haynes, E.N., Sucharew, H., Kuhnell, P., Alden, J., Barnas, M., Wright, R.O., Parsons, P. J., Aldous, K.M., Praamsma, M.L., Beidler, C., Dietrich, K.N., 2015. Manganese exposure and neurocognitive outcomes in rural school-age children: the communities actively researching exposure study (Ohio, USA). Environ. Health Perspect. 123, 1066–1071. https://doi.org/10.1289/ehp.1408993.

Hernández-Pellón, A., Fernández-Olmo, I., 2019a. Airborne concentration and deposition of trace metals and metalloids in an urban area downwind of a manganese alloy plant. Atmos. Pollut. Res. 10, 712–721. https://doi.org/10.1016/j.apr.2018.11.009.

Hernández-Pellón, A., Fernández-Olmo, I., 2019b. Using multi-site data to apportion PM-bound metal(loid)s: impact of a manganese alloy plant in an urban area. Sci. Total Environ. 651, 1476–1488. https://doi.org/10.1016/j.scitotenv.2018.09.261.

Hernández-Pellón, A., Nischkauer, W., Limbeck, A., Fernández-Olmo, I., 2018. Metal (loid) bioaccessibility and inhalation risk assessment: a comparison between an urban and an industrial area. Environ. Res. 165, 140–149. https://doi.org/10.1016/j.envres.2018.04.014.

Herrero-Montes, M., Alonso-Blanco, C., Paz-Zulueta, M., Sarabia-Cobo, C., Ruiz-Azcona, L., Parás-Bravo, P., 2019. Binge drinking in Spanish university students: associated factors and repercussions: a preliminary study. Int. J. Environ. Res. Public Health 16 (23), 4822. https://doi.org/10.3390/ijerph16234822.

Kastury, F., Smith, E., Juhasz, A.L., 2017. A critical review of approaches and limitations of inhalation bioavailability and bioaccessibility of metal(loid)s from ambient particulate matter or dust. Sci. Total Environ. 574, 1054–1074. https://doi.org/10.1016/j.scitotenv.2016.09.056.

Kastury, F., Smith, E., Karna, R.R., Scheckel, K.G., Juhasz, A.L., 2018. Methodological factors influencing inhalation bioaccessibility of metal(loid)s in PM2.5 using simulated lung fluid. Environ. Pollut. 241, 930–937. https://doi.org/10.1016/j.envpol.2018.05.094.

Kelly, F.J., Fussell, J.C., 2012. Size, source and chemical composition as determinants of toxicity attributable to ambient particulate matter. Atmos. Environ. 60, 504–526. https://doi.org/10.1016/j.atmosenv.2012.06.039.

Kenborg, L., Lassen, C.F., Hansen, J., Olsen, J.H., 2012. Parkinson's disease and other neurodegenerative disorders among welders: a Danish cohort study. Mov. Disord. 27 (10), 1283–1289. https://doi.org/10.1002/mds.25125.

Kim, Y., Bowler, R.M., Abdelouahab, N., Harris, M., Gocheva, V., Roels, H.A., 2011. Motor function in adults of an Ohio community with environmental manganese exposure. Neurotoxicology 32, 606–614. https://doi.org/10.1016/j.neuro.2011.07.011.

Lezak, M.D., 2004. Neuropsychological Assessment, 4th ed. Oxford University Press, New York.

Lin, C.C., Chen, Y.C., Su, F.C., Lin, C.M., Liao, H.F., Hwang, Y.H., Hsieh, W.S., Jeng, S.F., Su, Y.N., Chen, P.C., 2013. In utero exposure to environmental lead and manganese and neurodevelopment at 2 years of age. Environ. Res. 123, 52–57. https://doi.org/10.1016/j.envres.2013.03.003.

Lucas, E.L., Bertrand, P., Guazzetti, S., Donna, F., Peli, M., Jursa, T.P., Lucchini, R., Smith, D.R., 2015. Impact of ferromanganese alloy plants on household dust manganese levels: implications for childhood exposure. Environ. Res. 138, 279–290. https://doi.org/10.1016/j.envres.2015.01.019.

Lucchini, R., Apostoli, P., Perrone, C., Placidi, D., Albini, E., Migliorati, P., Mergler, D., Sassine, M.P., Palmi, S., Alession, L., 1999. Long-term exposure to "low levels" of manganese oxides and neurofunctional changes in ferroalloy workers. Neurotoxicology 20, 287–298.

Lucchini, R.G., Martin, C.J., Doney, B.C., 2009. From manganism to manganese induced parkinsonism: a conceptual model based on the evolution of exposure. Neuromol. Med. 11, 311–321. https://doi.org/10.1007/s12017-009-8108-8.

Lucchini, R.G., Guazzetti, S., Zoni, S., Donna, F., Peter, S., Zacco, A., Salmistraro, M., Bontempi, E., Zimmerman, N.J., Smith, D.R., 2012. Tremor, olfactory and motor changes in Italian adolescents exposed to historical ferro-manganese emission. Neurotoxicology 33, 687–696. https://doi.org/10.1016/j.neuro.2012.01.005.

Lucchini, R.G., Guazzetti, S., Zoni, S., Benedetti, C., Fedrighi, C., Peli, M., Donna, F., Bontempi, E., Borgese, L., Micheletti, S., Ferri, R., Marchetti, S., Smith, D.R., 2014. Neurofunctional dopaminergic impairment in elderly after lifetime exposure to manganese. Neurotoxicology 45, 309–317. https://doi.org/10.1016/j.neuro.2014.05.006.

Manjón, I., Ramírez-Andreotta, M.D., Sáez, A.E., Root, R.A., Hild, J., Janes, M.K., Alexander-Ozinskas, A., 2020. Ingestion and inhalation of metal(loid)s through preschool gardening: an exposure and risk assessment in legacy mining communities. Sci. Total Environ. 718. https://doi.org/10.1016/j.scitotenv.2019.134639.

Markiv, B., Ruiz-Azcona, L., Expósito, A., Santibáñez, M., Fernández-Olmo, I., 2020. Human whole blood, fingernails and hair as biomarkers of exposure to trace metals near an urban/industrial mixed area. In: 20th International Conference on Heavy Metals in the Environment (ICHMET). Seoul.

Mbengue, S., Alleman, L.Y., Flament, P., 2015. Bioaccessibility of trace elements in fine and ultrafine atmospheric particles in an industrial environment. Environ. Geochem. Health 37, 875–889. https://doi.org/10.1007/s10653-015-9756-2.

Menezes-Filho, J.A., Paes, C.R., Pontes, A.M., Moreira, J.C., Sarcinelli, P.N., Mergler, D., 2009. High levels of hair manganese in children living in the vicinity of a ferro-manganese alloy production plant. Neurotoxicology 30, 1207–1213. https://doi.org/10.1016/j.neuro.2009.04.005.

Mergler, D., Huel, G., Bowler, R., Iregren, A., Bélanger, S., Baldwin, M., Tardif, R., Smargiassi, A., Martin, L., 1994. Nervous system dysfunction among workers with long-term exposure to manganese. Environ. Res. 64, 151–180. https://doi.org/10.1006/enrs.1994.1013.

Mergler, D., Baldwin, M., Bélanger, S., Larribe, F., Beuter, A., Bowler, R., Panisset, M., Edwards, R., de Geoffroy, A., Sassine, M.P., Hudnell, K., 1999. Manganese neurotoxicity, a continuum of dysfunction: results from a community-based study. Neurotoxicology 20, 327–342.

Michalke, B., Fernsebner, K., 2014. New insights into manganese toxicity and speciation. J. Trace Elem. Med. Biol. 28, 106–116. https://doi.org/10.1016/j.jtemb.2013.08.005.

Mukherjee, A., Agrawal, M., 2017. World air particulate matter: sources, distribution and health effects. Environ. Chem. Lett. 15, 283–309. https://doi.org/10.1007/s10311-017-0611-9.

Mukhtar, A., Limbeck, A., 2013. Recent developments in assessment of bio-accessible trace metal fractions in airborne particulate matter: a review. Anal. Chim. Acta 774, 11–25.

Ntihabose, R., Surette, C., Foucher, D., Clarisse, O., Bouchard, M.F., 2018. Assessment of saliva, hair and toenails as biomarkers of low level exposure to manganese from drinking water in children. Neurotoxicology 64, 126–133. https://doi.org/10.1016/j.neuro.2017.08.011.

Oteo, J.A., Benavente, P., Garzón, M., 2015. Valores normativos de la fuerza de puño en la población española en edad laboral. Influencia de las variables antropométricas de la mano y el antebrazo. Rev. Iberoam. Cir. Mano 43 (2), 104–110. https://doi.org/10.1016/j.ricma.2015.09.005.

Pollitt, K.J.G., Maikawa, C.L., Wheeler, A.J., Weichenthal, S., Dobbin, N.A., Liu, L., Goldberg, M.S., 2016. Trace metal exposure is associated with increased exhaled nitric oxide in asthmatic children. Environ. Health 15 (1), 94. https://doi.org/10.1186/s12940-016-0173-5.

Racette, B.A., Searles Nielsen, S., Criswell, S.R., Sheppard, L., Seixas, N., Warden, M.N., Checkoway, H., 2017. Dose-dependent progression of parkinsonism in manganese-exposed welders. Neurology 88 (4), 344–351. https://doi.org/10.1212/WNL.0000000000003533.

Reis, A.P., Costa, S., Santos, I., Patinha, C., Noack, Y., Wragg, J., Cave, M., Sousa, A.J., 2015. Investigating relationships between biomarkers of exposure and environmental copper and manganese levels in house dusts from a Portuguese industrial city. Environ. Geochem. Health 37 (4), 725–744. https://doi.org/10.1007/s10653-015-9724-x.

Rodrigues, J.L.G., Araújo, C.F.S., dos Santos, N.R., Bandeira, M.J., Anjos, A.L.S., Carvalho, C.F., Lima, C.S., Abreu, J.N.S., Mergler, D., Menezes-Filho, J.A., 2018a. Airborne manganese exposure and neurobehavior in school-aged children living near a ferro-manganese alloy plant. Environ. Res. 167, 66–77. https://doi.org/10.1016/j.envres.2018.07.007.

Rodrigues, J.L.G., Bandeira, M.J., Araújo, C.F.S., dos Santos, N.R., Anjos, A.L.S., Koin, N. L., Pereira, L.C., Oliveira, S.S.P., Mergler, D., Menezes-Filho, J.A., 2018b. Manganese and lead levels in settled dust in elementary schools are correlated with biomarkers of exposure in school-aged children. Environ. Pollut. 236, 1004–1013. https://doi.org/10.1016/j.envpol.2017.10.132.

Roels, H., Lauwerys, R., Buchet, J., Genet, P., Sarhan, M.J., Hanotiau, I., de Fays, M., Bernard, A., Stanescu, D., 1987. Epidemiological survey among workers exposed to manganese: effects on lung, central nervous system, and some biological indices. Am. J. Ind. Med. 11, 307–327. https://doi.org/10.1002/ajim.4700110308.

Roth, J.A., 2006. Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination. Biol. Res. 39, 45–57. https://doi.org/10.4067/s0716-97602006000100006.

Rothman, K.J., 1986. Modern Epidemiology. Little, Brown and Company, Boston.

Ruiz-Azcona, L., Fernández-Olmo, I., Expósito, A., Markiv, B., Paz-Zulueta, M., Parás-Bravo, P., Sarabia-Cobo, C., Santibáñez, M., 2021. Impact of environmental airborne manganese exposure on cognitive and motor functions in adults: a systematic review and meta-analysis. Int. J. Environ. Res. Public Health 18 (8), 4075. https://doi.org/10.3390/ijerph18084075.

Soler-Blasco, R., Murcia, M., Lozano, M., González-Safont, L., Amorós, R., Ibarluzea, J., Broberg, K., Irizar, A., Lopez-Espinosa, M.J., Lertxundi, N., Marina, L.S., Ballester, F., Llop, S., 2020. Prenatal manganese exposure and neuropsychological development in early childhood in the INMA cohort. Int. J. Hyg. Environ. Health 224, 113443. https://doi.org/10.1016/j.ijheh.2019.113443.

Solís-Vivanco, R., Rodríguez-Agudelo, Y., Riojas-Rodríguez, H., Ríos, C., Rosas, I., Montes, S., 2009. Cognitive impairment in an adult Mexican population non-occupationally exposed to manganese. Environ. Toxicol. Pharmacol. 28 (2), 172–178. https://doi.org/10.1016/j.etap.2009.04.001.

Standridge, J.S., Bhattacharya, A., Succop, P., Cox, C., Haynes, E.N., 2008. Effect of chronic low-level manganese exposure on postural balance: a pilot study of residents in Southwest Ohio. J. Occup. Environ. Med. 50, 1421–1429. https://doi.org/10.1097/JOM.0b013e3181896936.

Strauss, E., Sherman, E.M.S., Spreen, O., 2006. A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary, 3th ed. Oxford University Press, New York.

U.S. Environmental Protection Agency (USEPA), 2007. Estimation of Relative Bioavailability of lead in soil and soil-like materials using in vivo and in vitro methods. Environ. Health Perspect. 8, 1162–1171. https://ehp.niehs.nih.gov/doi/pdf/10.1289/ehp.8852.

Viana, G.F.S., Carvalho, C.F., Nunes, L.S., Rodrigues, J.L.G., Ribeiro, N.S., Almeida, D.A., Ferreira, J.R.D., Abreu, N., Menezes-Filho, J.A., 2014. Noninvasive biomarkers of manganese exposure and neuropsychological effects in environmentally exposed adults in Brazil. Toxicol. Lett. 231, 169–178. https://doi.org/10.1016/j.toxlet.2014.06.018.

Weggeberg, H., Benden, T.F., Lierhagen, S., Steinnes, E., Flaten, T.P., 2019. Characterization and bioaccessibility assessment of elements in urban aerosols by extraction with simulated lung fluids. Environ. Chem. Ecotoxicol. 1, 49–60. https://doi.org/10.1016/j.enceco.2019.10.001.

Williams, M., Todd, G.D., Roney, N., Crawford, J., Coles, C., McClure, P.R., Garey, J.D., Zaccaria, K., Citra, M., 2012. Toxicological Profile for Manganese. Agency for Toxic Substances and Disease Registry (US ATSDR), Atlanta (GA).

Zheng, W., Fu, S.X., Dydak, U., Cowan, D.M., 2011. Biomarkers of manganese intoxication. Neurotoxicology 32, 1–8. https://doi.org/10.1016/j.neuro.2010.10.002.