atmosphere

MDPI

Article

Comparison of Exposure to Pb and Mn Levels by Using Environmental Personal Monitors and Biomarkers in Relation to Cognitive and Motor Function

Miguel Santibáñez [1,*], Laura Ruiz-Azcona [1], Andrea Expósito [2], Bohdana Markiv [2] and Ignacio Fernández-Olmo [2]

[1] Global Health Research Group, Dpto Enfermería, Faculty of Nursing, Universidad de Cantabria-IDIVAL, Avda. Valdecilla, s/n, 39008 Santander, Spain
[2] Dpto. de Ingenierías Química y Biomolecular, Universidad de Cantabria, Avda. Los Castros, s/n, 39005 Santander, Cantabria, Spain; ignacio.fernandez@unican.es (I.F.-O.)
* Correspondence: miguel.santibanez@unican.es

Abstract: We conducted a cross-sectional study of 130 participants living near a ferromanganese alloy plant, analyzing Pb and Mn exposure by biomarkers (blood, hair, and fingernails) and particulate matter personal environmental monitors (PEMs). Cognitive and motor function were assessed by five and three tests, respectively. Mean differences (MDs) adjusted for age, sex, and study level were determined. In addition, MDs for Pb were adjusted for Mn levels and vice versa. Medians of 9.14 µg/L, 149.04 ng/g, and 96.04 ng/g were obtained for blood, scalp hair, and fingernails Pb levels, respectively. Regarding PEMs, median Pb levels were 6.56 ng/m^3 for the fine fraction and, for the coarse fraction, they were below the limit of detection in 97% of participants. Exposure to Pb at low levels was not associated with worse cognitive function. In comparison, exposure to high levels of Mn was associated with worse cognitive function at least in the domains evaluated through Stroop, Digit Span, and Verbal Fluency tests. In terms of motor function, our results suggest that even the currently low Pb levels may have negative health effects on dynamometer-determined strength—adjusted MD on dominant hand = −2.68; 95%CI (−4.85 to −0.51), $p = 0.016$. Further studies should investigate this association.

Keywords: environmental exposure; lead; manganese; cognitive function; motor function; biomarker; PM

Citation: Santibáñez, M.; Ruiz-Azcona, L.; Expósito, A.; Markiv, B.; Fernández-Olmo, I. Comparison of Exposure to Pb and Mn Levels by Using Environmental Personal Monitors and Biomarkers in Relation to Cognitive and Motor Function. *Atmosphere* **2024**, *15*, 350. https://doi.org/10.3390/atmos15030350

Academic Editor: Maurice Millet

Received: 2 February 2024
Revised: 29 February 2024
Accepted: 5 March 2024
Published: 13 March 2024

1. Introduction

Various metals and metalloids present in airborne particulate matter (PM) are associated with health toxicity. For this reason, some of them are regulated in air quality legislations, setting a limit/target value calculated from the total metal content in PM [1–3]. Maximum values are also recommended by other institutions, such as the Agency for Toxic Substances and Disease Registry (ATSDR), which sets Minimal Risk Levels (MRLs) for some metals [4]. In addition, the US Environmental Protection Agency (EPA) uses a Reference Concentration (RfC) for some metals for the calculation of noncarcinogenic health risk. Regarding neurological effects, lead (Pb) is a well-known neurotoxic metal and a good example of regulated metal [2,3]. Because of these legislative actions, it is also well known that there has been a decrease in blood Pb in the general population in the last few decades, in both adults and children, mainly as a result of the banning of Pb additives in fuels [5,6], in addition to other older actions such as the removal of Pb from soldered cans [7]. In the EU, airborne Pb is regulated through Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe [2]; so, in member countries such as Spain, current blood Pb levels, even in mixed urban–industrial areas, are expected to be much lower than in previous decades.

Most of the studies analyzing the effect of Pb exposure on cognitive and motor function have used datasets from before 2010 with blood Pb levels with mean values higher than 30 μg/L. At these levels, most of these studies showed decrements in neurological function [4,8–13], mainly when some cumulative biomarkers were used as predictors [8,14–16]. However, ATSDR considers the lowest reported blood Pb levels to still be associated with serious adverse effects [4]. So, new studies analyzing the effects of the currently lower Pb levels are needed to reinterpret the safety of present Pb exposure levels.

In relation to Mn, recent studies and reviews have also highlighted its neurotoxic effects, associated with environmental airborne exposure, mainly on cognitive and motor function [17,18], even though it is an essential trace element found in the human body [19]. For Mn, the legislative situation is the opposite, since there is no limit/target value in European air quality legislation for this metal. However, the World Health Organization (WHO) proposed an annual guideline value of 150 ng/m^3 [20]. Likewise, the US EPA set an RfC of 50 ng/m^3, according to evaluation under the Integrated Risk Information System (IRIS) program [21]. Later, the California EPA proposed an annual reference exposure level for Mn of 90 ng/m^3 [22].

With this rationale, the BIONEUROMET study was carried out in northern Spain in the Bay of Santander, where the capital (Santander, 172,000 inhabitants) has been exposed to elevated levels of airborne Mn historically due to the presence of a ferromanganese alloy smelter in a nearby town called Maliaño (9535 inhabitants), usually exceeding the RfC given by the US EPA (i.e., 50 ng/m^3) [23–25]. In the BIONEUROMET study, PM personal samplers, also known as PM personal environmental monitors (PEMs), were used for each voluntary person in addition to stationary PM samplers, allowing for personal PM-bound metal sampling [26]. With these PEMs, both the non-bioaccessible and the bioaccessible concentrations of metals were determined for the fine and coarse fractions separately; this bioaccessible concentration may better represent the exposure risk [27–29]. In addition to PEMs, the personal characterization of exposure in the BIONEUROMET study was determined through biomarkers (blood, scalp hair, and fingernails).

Therefore, our objective was to analyze the impact of the currently low environmental Pb exposure in a mixed urban–industrial area on cognitive and motor function in adults, comparing these effects with those of high levels of airborne Mn in the same study population.

2. Methods

2.1. Design, Area of Study, and Participants

The characteristics of the study area (Bay of Santander, northern Spain) were previously reported [23,24]. Briefly, Santander is the capital and major city of the Cantabria region (about 172,000 inhabitants in 2022); although it is mainly characterized by commercial and service-based activities, the presence of some industrial sources in the southern part of the bay leads to moderate to high levels of some metals in ambient air, mainly in Maliaño, where a ferromanganese alloy plant with an annual production capacity of more than 100 kt of ferromanganese and silicomanganese is located. In a former work, the contribution of this plant to the total Mn emissions in the study area was estimated to be 91% [24]. As a typical Spanish northern coastline region, it has an Atlantic climate with mild temperatures and frequent rainfall. The prevailing wind direction in this area is SW; so, Maliaño and Santander are located downwind of the industrial source of Mn, leading to elevated airborne Mn levels [23–25]. Due to these high levels of Mn, this area was selected for the BIONEUROMET cross-sectional study; a detailed description of the recruitment process was previously published [30,31]. Overall, a final adult study population (n = 130) was obtained from the town of Maliaño (n = 65) and the rest of the bay (n = 65), mainly from the city of Santander, located between 5 and 10 km from this Industrial Emission Source (IES) of Mn. The location of the residence of the BIONEUROMET participants and the ferromanganese plant are found in Figure 1.

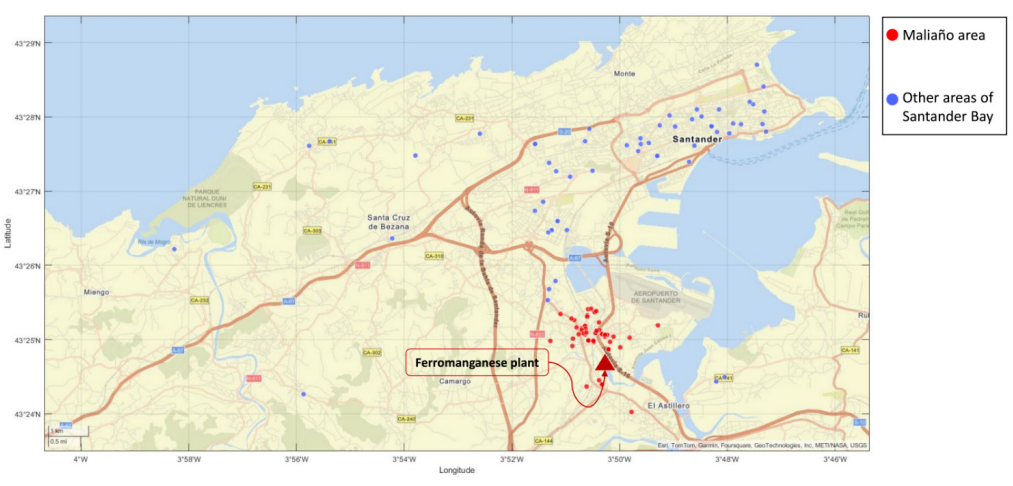

Figure 1. Location of the ferromanganese plant and residences of the BIONEUROMET participants.

This study was approved by the Research Ethics Committee of the University of Cantabria (CEUC) and the Clinical Research Ethics Committee in Cantabria (CEIC). Written informed consent was obtained from each participant.

2.2. Cognitive and Motor Function Tests and Data Collection

The details of data collection have been described elsewhere [30,31]. Each test was conducted in a standardized way in the same testing room by a single experienced investigator (L.R.-A). The following five cognitive function and three motor function tests were used.

The Stroop Color Word test consists of three parts. In the first part (Stroop Word), the words "RED", "GREEN", and "BLUE" are randomly written in black color within columns. The better the cognitive function, the more words are correctly read. In the second part (Stroop Color), these words have been replaced by "XXXX" printed randomly in red, green, and blue colors within the column. The more colors correctly said within 45 s, the better the cognitive function. In the third part (Color&Word), the words "RED", "GREEN", and "BLUE" are presented but printed in red, green, and blue colors instead of black. The more colors correctly named, the better the cognitive function [32].

The Digit Span consists of two parts. In the Forward section, participants are required to repeat sequences of numbers in the same order as read by the evaluator, while, in the Backward section, the sequences must be repeated in reverse order. The longer the sequence repeated correctly in each part, the better the scores and the cognitive function [33].

In the Verbal Fluency tests, the more words starting with P, M, and R letters a person was able to say within a 60-s timeframe, the better the cognitive function [34].

The Trail Making Test (TMT) consists of two parts. In the TMT-A, participants are instructed to draw a line sequentially linking the numbers 1 to 25 as fast as possible. In the TMT-B, participants have to draw a line by sequentially connecting numbers and letters, so it is a somewhat complicated task. In both parts, an increased duration to complete the task is associated with poorer cognitive function [35,36].

The Rey Osterrieth Complex Figure (ROCF) was the last cognitive test administered and it also consists of two parts. In the Copy part, participants had to copy the figure itself, while, in the Delay part, they were required to repeat the figure after a 30-min interval. The more parts copied and repeated correctly, the better the cognitive function [37].

The crude cognitive scores were standardized according to NEURONORMA norms [38] so that, even for TMT, the higher the NEURONORMA scores, the better the cognitive function.

In the Finger Tapping test (FTT), the number of taps for the dominant and nondominant hand was recorded by a counter for 10 s (WPS electronic Tapping Test). The higher the number of taps, the better the motor function [39].

In the Grooved Pegboard test, participants were required to insert pegs with ridges into a 5 × 5 matrix of holes using both the dominant and nondominant hands (Model 32025

of Lafayette Instrument Company (Lafayette, IN, USA)). The longer the time (seconds) taken to complete the task, the poorer the motor function [40].

In the dynamometer test, each participant had to squeeze twice (T.K.K. 5401 Grip-D, Takei, Tokyo, Japan), and the highest scores from the two measurements were selected for each dominant and nondominant hand. Better motor function is denoted by higher grip strength in kilograms [41].

At the end of the cognitive and motor function tests, the subject was required to carry with him/her a PEM for at least 24 h. The following day, each participant handed over the PEM, after which the biological samples were collected.

2.3. Biomarker Sampling and Analysis

Whole blood samples (7.5 mL) were collected by using lithium heparin monovettes developed for metal determinations (Sarstedt, Nümbrecht, Germany), being stored at 4 °C for a maximum of 14 days until analysis. A tuft of hair of approximately 0.5 g (from the 2 cm closest to the occipital part of the scalp) was cut using ceramic scissors (Kyocera advanced ceramics CS-124).

Fingernails were obtained from both hands (previously washed with a liquid soap) using nail clippers. They were stored in sterile propylene bottles until analysis and were washed exhaustively in the laboratory, removing all exogenous metals before digestion, following the procedure described by Eastman et al. [42]. Additional information on pre-analytical conditions and the ICP-MS determinations (Agilent 7500 CE) is available in Markiv et al. [43].

The limit of detection (LOD) was 1.48 µg/L for blood Pb, with 0.8% of samples being below this LOD. For fingernails, LODs were 0.85–29.12 ng/g and 2.46–22.49 ng/g for scalp hair, with all Pb determinations above them. With regards to Mn, LODs were 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, with all Mn determinations being above them; see Table 1.

Table 1. Limits of detection (LOD) for Pb and Mn and percentage of samples analyzed below the LOD.

Matrix	Pb (LOD)	Pb (% < LOD)	Mn (LOD)	Mn (% < LOD)
Blood (µg/L)	1.48	0.8	0.74	0
Scalp hair (ng/g)	0.85–29.12	0	3.37–115.86	0
Fingernails (ng/g)	2.46–22.49	0	9.76–89.23	0
$PM_{10\text{-}2.5}$ bioaccessible (ng/m^3)	5.74	97.7	0.76	1.5
$PM_{10\text{-}2.5}$ non-bioaccessible (ng/m^3)	1.84	96.9	2.52	40.8
$PM_{2.5}$ bioaccessible (ng/m^3)	0.42	12.3	0.59	4.6
$PM_{2.5}$ non-bioaccessible (ng/m^3)	0.73	53.1	0.99	6.9

2.4. PEM Sampling and Analysis

Portable impactors (SKC PMI coarse) connected to a personal pump (SKC Aircheck XR5000) calibrated at a flow rate of 3 L/min were used for PEM sampling. The fine ($PM_{2.5}$) and coarse ($PM_{10\text{-}2.5}$) modes were separately collected on 37 and 25 mm PTFE membrane filters, and then the Pb and Mn levels for the bioaccessible and non-bioaccessible fractions were determined following a two-step procedure developed by Expósito et al. [25].

The LODs for Pb are also shown in Table 1 (5.74, 1.84, 0.42, and 0.73 ng/m^3). The percentage of samples below these LODs was 97.7, 96.9, 12.3, and 53.1% for the bioaccessible and the non-bioaccessible concentrations of $PM_{10\text{-}2.5}$ and $PM_{2.5}$, respectively. For Mn, the LODs were 0.76, 2.52, 0.59, and 0.99 ng/m^3 with 1.5, 40.8, 4.6, and 6.9% of samples below them, respectively.

2.5. Statistical Analysis

Categorical and discrete variables were described as percentages. Statistical differences between groups were compared by using the Chi-square test with Yates' correction or Fisher's exact test when appropriate. Continuous variables were expressed as mean and standard deviation (SD) and/or median and interquartile ranges (IQR). The Student's T-test (for equal or different variances as a function of results in the Levene test) was used for mean comparisons, and the Mann–Whitney U test was used for the comparisons of medians.

For values <LOD, ½ of the corresponding LOD value was assigned. Regarding Pb levels of PEMs and cognitive and motor function results, due to the high number of values <LOD for Pb in the coarse fraction (>97%), only associations for the fine fraction ($PM_{2.5}$) were estimated.

Lead and Mn exposure was dichotomously categorized (0 = lower values; 1 = higher values) according to the median, and adjusted mean differences (MDs) with their 95% confidence intervals (CI) were estimated by using linear regression models, incorporating as dependent variables in each model the quantitative results in each cognitive or motor function test and with age, sex, and educational level treated as confounders. Likewise, $PM_{2.5}$, blood, scalp hair, and fingernails Pb exposures were adjusted for $PM_{2.5}$, blood, scalp hair, and fingernails Mn levels, respectively, and vice versa (Mn levels were adjusted for Pb levels). SPSS package 24.0 (SPSS, Inc., Chicago, IL, USA) was used as statistical software. All tests were two-tailed, with an alpha error of 0.05.

3. Results

3.1. Basal Characteristics and Levels of Pb and Mn in the Study Population

The mean age of the study participants was 41.72 years (SD 13.97), with 73.1% (95/130) being women. Regarding the study level, 55.4% had university studies, 23.1% had high school education, 16.9% had secondary school education, and 4.6% had primary education. The mean number of years residing at the same last address was 15.26 years (SD 13.71), and 74.6% of the participants were full-time employed; see Supplementary Table S1.

Table 2 shows Pb and Mn concentrations in biomarkers and PEM sampling as a function of gender. Medians of 9.14 μg/L, 149.04 ng/g, and 96.04 ng/g were obtained for blood, scalp hair, and fingernails Pb levels, respectively (see Table 2 and Figure S1). The median of total Pb concentration (sum of bioaccessible and non-bioaccessible) was 6.56 ng/m^3 for the fine fraction. Regarding blood, scalp hair, and fingernails Mn levels, medians of 9.58 μg/L, 185.31 ng/g, and 555.28 ng/g were obtained, respectively. With respect to total Mn in PM_{10} (sum of bioaccessible and non-bioaccessible coarse and fine fractions), a mean of 151.9 (SD 331.66) and a median of 43.87 ng/m^3 were obtained.

Table 2. Pb and Mn concentrations in biomarkers (blood, scalp hair, and fingernails) and personal sampling (bioaccessible, non-bioaccessible, and total) in the whole study population and as a function of gender.

	Women			**Men**			**Total**			
	Mean (SD)	**Median**	**P_{95}**	**Mean (SD)**	**Median**	**P_{95}**	**Mean (SD)**	**Median**	**P_{95}**	***p* Value ***
Biomarker sampling										
Blood (μg/L)										
Pb (μg/L)	9.74 (5.52)	8.49	19.52	14.53 (9.17)	11.4	40.24	11.03 (6.99)	9.14	24.82	*0.001*
Mn (μg/L)	10.04 (3.14)	9.76	15.89	9.71 (4.01)	9.01	18.51	9.95 (3.38)	9.58	16.01	*0.375*
Scalp hair (ng/g)										
Pb (ng/g)	210.9 (214.6)	139	669.5	361.5 (368.7)	234	1387.2	246.7 (265.7)	149.04	861.1	*0.007*
Mn (ng/g)	220.5 (205.4)	168.3	721.7	366.3 (427.2)	295.9	1550.5	255.2 (279.6)	185.31	719	*0.008*
Fingernails (ng/g)										
Pb (ng/g)	127.5 (208.5)	97.3	402	128.5 (119.3)	94.3	474.1	150.5 (190.8)	96.04	424.8	0.866
Mn (ng/g)	967.5 (1097.1)	562	3778	844.3 (1203.3)	532	4331	936.7 (1120.5)	555.28	3549.2	*0.337*

Table 2. *Cont.*

	Women			Men			Total			
	Mean (SD)	**Median**	**P_{95}**	**Mean (SD)**	**Median**	**P_{95}**	**Mean (SD)**	**Median**	**P_{95}**	***p* Value ***
PEM sampling $PM_{2.5}$ (ng/m^3)										
Pb bioaccessible $PM_{2.5}$ (ng/m^3)	13.24 (21.10)	4.5	54.8	11.86 (13.71)	7.4	49.38	12.87 (19.34)	5.25	50.09	*0.634*
Pb non-bioaccessible $PM_{2.5}$ (ng/m^3)	1.86 (2.80)	0.37	6.05	0.90 (1.01)	0.37	3.85	1.60 (2.48)	0.37	5.61	*0.081*
Pb total $PM_{2.5}$ (ng/m^3)	15.10 (22.82)	5.87	56.10	12.76 (14.06)	9.1	50.65	14.47 (20.80)	6.56	51.19	*0.877*
Mn bioaccessible $PM_{2.5}$ (ng/m^3)	70.07 (147.06)	17.82	323.9	53.12 (100.09)	12.85	298.6	66.31 (135.79)	17.05	315.5	*0.68*
Mn non-bioaccessible $PM_{2.5}$ (ng/m^3)	14.02 (23.27)	5.73	66.41	8.91 (9.98)	6.61	33.28	12.64 (20.64)	5.8	61.23	*0.858*
Mn total $PM_{2.5}$ (ng/m^3)	84.08 (159.31)	25.26	355.6	65.03 (102.57)	20.02	308.1	78.95 (146.07)	25	350.6	*0.937*
$PM_{10\text{-}2.5}$ (ng/m^3)										
Mn bioaccessible $PM_{10\text{-}2.5}$ (ng/m^3)	68.18 (204.49)	15.17	246.8	41.92 (72.82)	10.57	301	61.11 (178.90)	13.61	249.1	*0.527*
Mn non-bioaccessible $PM_{10\text{-}2.5}$ (ng/m^3)	12.73 (43.07)	3.4	42.13	9.36 (12.16)	2.62	39.72	11.82 (37.33)	3.39	39.73	*0.862*
Mn total $PM_{10\text{-}2.5}$ (ng/m^3)	80.91 (246.81)	16.51	287.1	51.28 (82.10)	15.46	340.7	79.93 (215.26)	16.47	289.2	*0.723*
PM_{10} (ng/m^3)										
Mn total PM_{10} (ng/m^3)	165.00 (376.19)	42.13	608.9	116.31 (155.90)	51.53	548.9	151.89 (331.66)	43.87	577.2	*0.927*

SD = standard deviation. P95 = 95 percentile. * Mann–Whitney U test. PEM = personal environmental monitor.

3.2. Associations between Pb and Mn Levels and Cognitive Function Results

Regarding Pb levels, non-statistically significant MDs were observed in all of the cognitive tests analyzed (Stroop, Digit Span, Verbal Fluency, TMT, and ROCF tests) except for blood Pb levels and Stroop Color part. In general, MDs were mixed (positive and negative but of small magnitude) or close to zero (null hypothesis), with the exception mentioned above of blood Pb levels and Stroop Color, for which a positive MD of borderline significance (indicating better cognitive function) was obtained for those with higher blood Pb levels; adjusted MD = +1.00; 95%CI (0.02 to 1.99), $p = 0.046$; see Tables 3, 4 and S2–S4.

In contrast, statistically significant lower scores (indicating worse cognitive function) were obtained in participants with higher levels of Mn in fingernails across all Stroop parts as follows: adjusted MD for Stroop Word part = −1.19; 95%CI (−2.30 to −0.09), $p = 0.034$; adjusted MD for Stroop Color part = −1.12; 95%CI (−2.18 to −0.07), $p = 0.037$; and adjusted MD for Stroop Color&Word part = −1.33; 95%CI (−2.48 to −0.17), $p = 0.025$ (See Table 3). Statistically significant negative MDs were also obtained for the two Digit Span parts as follows: adjusted MD for Digit Span Forward = −1.62; 95%CI (−2.77 to −0.47), $p = 0.006$; adjusted MD for Digit Span Backward = −1.78; 95%CI (−2.75 to −0.82), $p < 0.001$ (See Table 4). In addition, negative MDs were also obtained homogeneously according to PEMs for both bioaccessible and non-bioaccessible fractions, to a greater extent for Stroop (See Table 3) and Verbal Fluency test, in some cases yielding statistical significance (See Supplementary Table S2). For the rest of the cognitive function tests (TMT and ROCF), non-statistically or borderline negative MDs were obtained; see Supplementary Tables S3 and S4.

Table 3. Mean differences for Stroop Color Word test according to Pb and Mn levels in biomarkers and PM personal sampling.

	MD *	95%	CI	*p* Value
Biomarkers				
Pb Blood (>9.14 vs. ≤9.14 μg/L)				
Stroop Word	0.32	−0.71	1.35	*0.538*
Stroop Color	**1.00**	0.02	1.99	***0.046***
Stroop Color Word	−0.02	−1.10	1.06	*0.970*
Pb Scalp hair (>149.04 vs. ≤149.04 ng/g)				
Stroop Word	0.71	−0.22	1.63	*0.132*
Stroop Color	0.86	−0.08	1.80	*0.071*
Stroop Color Word	0.43	−0.57	1.43	*0.400*
Pb Fingernails (96.04> vs. ≤96.04 ng/g)				
Stroop Word	−0.39	−1.49	0.70	*0.478*
Stroop Color	−0.53	−1.59	0.52	*0.317*
Stroop Color Word	0.03	−1.14	1.20	*0.958*
Mn Blood (>9.58 vs. ≤9.58 μg/L)				
Stroop Word	0.71	−0.23	1.65	*0.138*
Stroop Color	0.78	−0.14	1.69	*0.094*
Stroop Color Word	0.90	−0.08	1.88	*0.071*
Mn Scalp hair (>185.31 vs. ≤185.31 ng/g)				
Stroop Word	−0.47	−1.42	0.48	*0.330*
Stroop Color	−0.52	−1.49	0.45	*0.289*
Stroop Color Word	−0.32	−1.35	0.70	*0.533*
Mn Fingernails (>555.28 vs. ≤555.28 ng/g)				
Stroop Word	**−1.19**	−2.30	−0.09	***0.034***
Stroop Color	**−1.12**	−2.18	−0.07	***0.037***
Stroop Color Word	**−1.33**	−2.48	−0.17	***0.025***
PEM Fine fraction ($PM_{2.5}$)				
Pb Bioaccessible (5.26+ vs. ≤5.25 ng/m^3)				
Stroop Word	0.06	−0.92	1.05	*0.900*
Stroop Color	−0.30	−1.28	0.68	*0.547*
Stroop Color Word	−0.20	−1.25	0.85	*0.702*
Pb Non-bioaccessible (0.38+ vs. ≤0.37 ng/m^3)				
Stroop Word	0.61	−0.37	1.59	*0.220*
Stroop Color	0.03	−0.94	1.00	*0.950*
Stroop Color Word	0.06	−0.98	1.11	*0.903*
Pb Total (Bio + Non-bio) (6.57+ vs. ≤6.56 ng/m^3)				
Stroop Word	0.32	−0.67	1.31	*0.520*
Stroop Color	−0.18	−1.16	0.81	*0.721*
Stroop Color Word	−0.32	−1.37	0.73	*0.545*
Mn Bioaccessible (17.06+ vs. ≤17.05 ng/m^3)				
Stroop Word	−0.71	−1.70	0.27	*0.156*
Stroop Color	−0.73	−1.71	0.24	*0.138*
Stroop Color Word	**−1.38**	−2.41	−0.36	***0.008***
Mn Non-bioaccessible (5.81+ vs. ≤5.80 ng/m^3)				
Stroop Word	−0.11	−1.05	0.84	*0.827*
Stroop Color	**−1.19**	−2.11	−0.27	***0.011***
Stroop Color Word	−0.72	−1.72	0.27	*0.154*
Mn Total (Bio + Non-bio) (25.01+ vs. ≤25.00 ng/m^3)				
Stroop Word	−0.93	−1.93	0.06	*0.065*
Stroop Color	−0.75	−1.74	0.23	*0.132*
Stroop Color Word	**−1.23**	**−2.27**	−0.18	***0.022***

MD = mean difference adjusted for age, sex, educational level, and Pb or Mn levels, respectively. PEM = personal environmental monitor. * A negative MD indicates worse cognitive function (lower standardized NEURONORMA scores) among those exposed to higher Pb or Mn levels. Statistically significant MDs are in bold.

Table 4. Mean differences for Digit Span test according to Pb and Mn levels in biomarkers and PM personal sampling.

	MD *	95%	CI	*p* Value
Biomarkers				
Pb Blood (>9.14 vs. ≤9.14 μg/L)				
Digit Span Forward	0.32	−0.70	1.34	*0.533*
Digit Span Backward	0.39	−0.50	1.27	*0.386*
Pb Scalp hair (>149.04 vs. ≤149.04 ng/g)				
Digit Span Forward	−0.47	−1.41	0.47	*0.324*
Digit Span Backward	0.48	−0.36	1.31	*0.259*
Pb Fingernails (96.04> vs. ≤96.04 ng/g)				
Digit Span Forward	−0.66	−1.83	0.51	*0.264*
Digit Span Backward	0.68	−0.29	1.64	*0.165*
Mn Blood (>9.58 vs. ≤9.58 μg/L)				
Digit Span Forward	−0.12	−1.06	0.81	*0.795*
Digit Span Backward	0.34	−0.48	1.16	*0.410*
Mn Scalp hair (>185.31 vs. ≤185.31 ng/g)				
Digit Span Forward	**−1.12**	−2.06	−0.18	***0.020***
Digit Span Backward	−0.62	−1.47	0.23	*0.153*
Mn Fingernails (>555.28 vs. ≤555.28 ng/g)				
Digit Span Forward	**−1.62**	−2.77	−0.47	***0.006***
Digit Span Backward	**−1.78**	−2.75	−0.82	***<0.001***
PEM Fine fraction ($PM_{2.5}$)				
Pb Bioaccessible (>5.25 vs. ≤5.25 ng/m^3)				
Digit Span Forward	−0.62	−1.60	0.36	*0.213*
Digit Span Backward	−0.02	−0.89	0.85	*0.966*
Pb Non-bioaccessible (>0.37 vs. ≤0.37 ng/m^3)				
Digit Span Forward	−0.64	−1.60	0.33	*0.196*
Digit Span Backward	0.03	−0.83	0.89	*0.942*
Pb Total (Bio + Non-bio) (>6.56 vs. ≤6.56 ng/m^3)				
Digit Span Forward	−0.62	−1.60	0.37	*0.216*
Digit Span Backward	0.18	−0.70	1.05	*0.691*
Mn Bioaccessible (>17.05 vs. ≤17.05 ng/m^3)				
Digit Span Forward	0.139	−0.832	1.11	*0.778*
Digit Span Backward	−0.156	−1.013	0.701	*0.719*
Mn Non-bioaccessible (>5.80 vs. ≤5.80 ng/m^3)				
Digit Span Forward	−0.542	−1.479	0.396	*0.255*
Digit Span Backward	−0.217	−1.041	0.608	*0.604*
Mn Total (Bio + Non-bio) (>25.00 vs. ≤25.00 ng/m^3)				
Digit Span Forward	0.157	−0.826	1.139	*0.753*
Digit Span Backward	−0.196	−1.064	0.672	*0.655*

MD = mean difference adjusted for age, sex, educational level, and Pb or Mn levels, respectively. PEM = personal environmental monitor. * A negative MD indicates worse cognitive function (lower standardized NEURONORMA scores) among those exposed to higher Pb or Mn levels. Statistically significant MDs are in bold.

3.3. Associations between Pb and Mn Levels and Motor Function Results

As regards Pb levels, non-statistically significant mixed MDs of small or null magnitude were also obtained for the Grooved Pegboard and FTT tests (see Supplementary Tables S5 and S6). Interestingly, negative MDs, indicating worse motor function (lower grip strength), were obtained for both dominant and nondominant hands in all biomarkers in relation to the dynamometer test (kg), yielding statistical significance for the nondominant hand as follows: adjusted MD for Pb levels = −2.68; 95%CI (−4.85 to −0.51), $p = 0.016$ (See Table 5). Concerning PEMs, non-statistically significant mixed MDs were obtained for all motor function tests; see Tables 5, S5 and S6.

Table 5. Mean differences for the dynamometer test (kg) according to Pb and Mn levels in biomarkers and PM personal sampling in dominant and nondominant hands.

	MD *	95%	CI	*p* Value
Biomarkers				
Pb Blood (>9.14 vs. ≤9.14 μg/L)				
dom hand	−1.59	−3.64	0.46	*0.127*
non-dom hand	−2.68	−4.85	−0.51	*0.016*
Pb Scalp hair (>149.04 vs. ≤149.04 ng/g)				
dom hand	−0.26	−2.12	1.59	*0.781*
non-dom hand	−0.47	−2.54	1.61	*0.657*
Pb Fingernails (96.04> vs. ≤96.04 ng/g)				
dom hand	−1.19	−3.53	1.14	*0.312*
non-dom hand	−1.30	−3.61	1.01	*0.267*
Mn Blood (>9.58 vs. ≤9.58 μg/L)				
dom hand	0.74	−1.14	2.61	*0.44*
non-dom hand	−0.13	−2.12	1.86	*0.895*
Mn Scalp hair (>185.31 vs. ≤185.31 ng/g)				
dom hand	0.44	−1.46	2.33	*0.651*
non-dom hand	0.19	−1.93	2.30	*0.861*
Mn Fingernails (>555.28 vs. ≤555.28 ng/g)				
dom hand	−0.62	−2.96	1.73	*0.603*
non-dom hand	0.07	−2.26	2.40	*0.953*
PEM Fine fraction ($PM_{2.5}$)				
Pb Bioaccessible (>5.25 vs. ≤5.25 ng/m^3)				
dom hand	0.02	−2.01	2.05	*0.986*
non-dom hand	−0.02	−2.19	2.15	*0.986*
Pb Non-bioaccessible (>0.37 vs. ≤0.37 ng/m^3)				
dom hand	0.81	−1.17	2.79	*0.420*
non-dom hand	0.33	−1.80	2.45	*0.762*
Pb Total (Bio + Non-bio) (>6.56 vs. ≤6.56 ng/m^3)				
dom hand	0.23	−1.81	2.26	*0.826*
non-dom hand	0.19	−1.98	2.37	*0.860*
Mn Bioaccessible (>17.05 vs. ≤17.05 ng/m^3)				
dom hand	0.98	−1.01	2.96	*0.332*
non-dom hand	0.81	−1.32	2.94	*0.454*
Mn Non-bioaccessible (>5.80 vs. ≤5.80 ng/m^3)				
dom hand	−1.37	−3.27	0.53	*0.157*
non-dom hand	−0.13	−2.18	1.93	*0.902*
Mn Total (Bio + Non-bio) (>25.00 vs. ≤25.00 ng/m^3)				
dom hand	1.14	−0.86	3.15	*0.260*
non-dom hand	1.30	−0.85	3.44	*0.235*

MD = mean difference adjusted for age, sex, educational level, and Pb or Mn levels, respectively. Dom = dominant. Non-dom = nondominant. PEM = personal environmental monitor. * A negative MD indicates worse motor function (lower grip strength) among those exposed to higher Pb or Mn levels.

Regarding Mn levels, non-statistically significant higher times in the Grooved Pegboard test and lower numbers of finger taps in FTT (indicating worse motor function) were observed for both hands in participants with higher Mn fingernails levels (see Supplementary Tables S5 and S6), whereas, for the dynamometer test, a negative non-significant MD (indicating also worse function with lower grip strength) was only observed for the dominant hand (See Table 5). For scalp hair Mn levels, mixed or positive (contrary to our hypothesis) non-significant MDs were obtained. For blood Mn levels, a lower time to complete the Grooved Pegboard test (indicating better motor function, contrary to our hypothesis) was observed, yielding statistical significance for the dominant hand as follows: adjusted MD = −2.82; 95%CI (−5.46 to −0.19), $p = 0.036$. Positive MDs indicating worse motor function (higher time to complete the Grooved Pegboard test) were obtained for both dominant and nondominant hands according to PEMs, reaching statistical significance for the bioaccessible fraction and the dominant hand as follows: adjusted MD =+3.36; 95%CI

(0.60 to 6.11), $p = 0.018$, whereas non-statistically significant mixed MDs were obtained for the rest of motor function tests; see Tables 5, S5 and S6.

4. Discussion

As mentioned in the methods section, Pb was mainly detected in the fine fraction ($PM_{2.5}$), where 88% and 47% of the determinations were above the LOD for the bioaccessible and non-bioaccessible fractions, respectively, with mean Pb concentrations of 12.87 and 1.60 ng/m^3. In the coarse fraction, determinations were lower than our LODs of 5.74 and 1.84 ng/m^3 for the bioaccessible and non-bioaccessible fractions in more than 97% of the participants. Thus, in a hypothetical sum of the Pb bioaccessible and non-bioaccessible concentrations obtained in the fine and coarse fractions to obtain the total concentration contained in PM_{10}, the overall result would be clearly below the annual PM_{10} limit value (500 ng/m^3) set by Directive 2008/50/EC [2]. These results agree well with those previously found in the same area from stationary PM samplers [23], reaching Pb levels between 6.91 and 15.6 ng/m^3 in PM_{10}.

The situation for Mn would be quite the opposite. The arithmetic mean of PM_{10}-bound Mn in the PEMs for the study population was 151.9 ng/m^3, above the WHO-recommended total air Mn value of 150 ng/m^3, and was far exceeded in participants residing in the vicinity of the IES of Mn, where the mean was 253.4 ng/m^3, rather similar to the annual average of 231.8 ng/m^3 measured in 2015 by a stationary sampler [23]. Comparing our Mn levels with other studies using PEMs, Mn levels found in our population are notably higher [44,45]. Our Mn levels are even higher than those of other studies carried out in residential areas, close to other Mn IES such as in Italy, where a mean value of 49.5 ng/m^3 in $PM_{2.5}$ has been reported in a town close to a ferromanganese alloy manufacturing plant [46], or in the US, where a geometric mean of 8.1 ng/m^3 in PM_{10} has been reported in Marietta (Ohio), located less than 10 km from the main ferromanganese plant of this country [47]. To our knowledge, higher Mn levels in PEMs have only been found in residences near Mn ore mines in Molango (Mexico), where a mean of 420 ng/m^3 in PM_{10} has been reported [48].

In this sense, our work has aimed to perform a comparative analysis between Pb and Mn exposure and its association with cognitive and motor function, taking advantage of the existence of the same population with low exposure to Pb but moderate or high exposure to Mn.

Our standardized cognitive function test battery included five tests assessing specifically attention, executive function, memory, and verbal fluency [49]. The Stroop Color Word test is considered a measure of executive function and cognitive flexibility [32]. The Digit Span, from the third version of the Wechsler Adult Intelligence Scale (WAIS III), is a measure of attention and working memory [33]. The Verbal Fluency test evaluates verbal association fluency as it computes the total number of words that a subject is able to say during 60 s [34]. TMT is a measure of attention, speed, and mental flexibility [35,36]. Finally, the purpose of ROCF is to assess visual–spatial constructional ability and visual memory [37]. Our motor function battery included three tests assessing specifically "eye-hand coordination and motor speed", "self-directed manual speed", and "grip strength" through the FTT, the Grooved Pegboard test, and the hand dynamometer test, respectively [49].

Our results do not suggest an association between the current Pb levels and poorer cognitive function in the context of a population with low Pb exposure. However, they do open the door to a possible negative effect (despite the low values) on motor function, as our participants with levels above our median blood Pb exposure (>9.14 μg/L) had lower mean dynamometer force, reaching statistical significance for the nondominant hand. The fact that our results were adjusted for age, sex, educational level, and even blood Mn levels would support the internal validity of our results; so, further studies should investigate the health safety of the currently low Pb levels in mixed urban–industrial settings.

With regard to exposure to higher levels of Mn in this case, our results, also adjusted for Pb levels as well as for sex, age, or educational level of the participants, support worse cognitive function at least in the domains determined by the Stroop, Digit Span

Backward, and Verbal Fluency test. For motor function, the evidence of association would be lower [30,31].

Manganese and Pb are two well-known neurotoxic trace metals, even at environmentally relevant concentrations. In particular, the basis of the derivation of the RfC from Mn used in the US EPA procedure for noncarcinogenic health risk assessment is the impairment of neurobehavioral function. In the case of Pb, no RfC is available in the US EPA procedure because it was not evaluated under the IRIS program. However, the ATSDR considers Pb as a neurotoxic metal that negatively affects neurological endpoints, even at relatively low exposure levels, measured as blood Pb concentrations (<100 μg/L). Moreover, MRLs for Pb have not been derived by the ATSDR because the lowest reported blood Pb levels are still associated with serious adverse effects (e.g., declining cognitive function in children). In adults, a large number of studies showing decrements in neurological function have been published, finding neurobehavioral effects in populations with blood Pb levels ≤100 μg/L, including decreased cognitive function, altered behavior and mood, and altered neuromotor and neurosensory function [4].

However, a more detailed analysis of this literature offers some controversial results regarding the effect of Pb on cognitive and motor endpoints, mainly at relatively low exposures. Thus, the cognitive function seems to be negatively affected in subjects containing low blood Pb levels only when some cumulative biomarkers are used as predictors, such as Pb in the tibia; in fact, the cognitive functions studied were not statistically significantly associated with blood Pb levels in most of these studies [8,14,15], probably because it provides integrated measures of exposure over shorter timeframes [9]. The main cognitive domains affected by the exposure to low Pb levels were language (including letter fluency), processing speed, executive functioning (including TMT-A and Stroop Color), verbal memory and learning, visual memory, visuoconstruction [14], visuospatial/visuomotor domains [10], story memory, category fluency [9], and working memory [11].

Whereas cognitive effects are better described in the literature, less information is available about motor functions in the general population at the currently lower exposure levels [50]. Former research, based on relatively high levels of blood Pb, on the effect of Pb exposure on fine motor function mainly assessed using a pegboard task showed significant associations with blood Pb, both in occupational and environmental studies [12,13,51–54]. However, later investigations found worse scores in the Grooved Pegboard test only when cumulative Pb exposure biomarkers were used, such as tibia and patella [16]. Bleecker et al. [12] also reported a higher effect size in the Grooved Pegboard test when the working lifetime weighted integrated blood lead (IBL) was used instead of the blood Pb but, in this study, blood levels were high (mean of 290 μg/L) because the study population was smelters exposed to airborne Pb. Less information was found on other fine motor tests, such as FTT. Casjens et al. [50] found that the fine-motor tests studied were not affected by elevated blood Pb, except for FTT in elderly men, with a weak effect for relatively high blood Pb concentrations (≥50 μg/L).

As mentioned in the introduction section, most of these cognitive and motor function studies were based on relatively old datasets (before 2010) and, therefore, the blood Pb levels in these studies were higher than the current levels. Our arithmetic mean blood Pb was 11.03 μg/L (median of 9.14 μg/L), clearly lower than in the majority of published studies, where typical mean values were > 30 μg/L, but similar to the current values in the general US population, with a median of 8.8 μg/L in 2015–2016 for those aged 20+ years [55]. The continuous decrease in blood Pb levels since the late 1970s has been reported in the literature [5,6]. Although the decline in the last two decades is smaller, it is still important, as observed in Figure S2 of the Supplementary Material. Thus, the meaning of low-level exposure to Pb has changed and the main conclusions derived from older studies should be interpreted carefully. In contrast to Pb, the case of Mn is different, as its level in some mixed urban–industrial areas remains high enough to potentially affect cognitive and motor function.

Regarding other methodological aspects of our study and limitations, in addition to confounding, the use of the same procedures and schedules for all participants by a single trained investigator with the same order of tests under the same conditions aimed to avoid any differential misclassification to minimize other potential biases. Since a high number of comparisons were made in our study, one limitation would be related to a multiple-test concern, so it cannot be entirely ruled out that some associations are actually false-positive results due to chance. We conducted a cross-sectional study, with the inherent lack of longitudinal data on both Pb and Mn exposure and neurological determinations as another limitation. Consequently, further cross-sectional and follow-up studies are needed to support the consistency of our results. Lastly, Fe, Zn, Cu, As, and Cd were also determined in PM personal samplers (bioaccessible and non-bioaccessible fractions) and biomarkers in our study. No associations for these metal(loid)s were found with our neurological test results in our independent analyses. It was expected, since only Pb and Mn are known neurotoxins among our studied metal(loid)s, so a crossover effect due to exposure to multiple simultaneous metal(loid)s does not seem to apply.

5. Conclusions

Lead exposure at the currently low levels in a mixed urban–industrial environment does not seem to be associated in our study with worse cognitive function. In comparison, exposure to high levels of Mn appears to be associated with worse cognitive function, at least in the domains evaluated through Stroop, Digit Span, and Verbal Fluency tests. In terms of motor function, our results suggest that even the currently low Pb levels may have negative health effects on dynamometer-determined strength. Further studies should investigate this association.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/atmos15030350/s1, Figure S1: Boxplots with the distribution of Pb values in analyzed biomarkers. Figure S2: Decrease in blood Pb levels in the US general population from 1999 to 2016 (calculated from the Fourth National Report on Human Exposure to Environmental Chemicals (U.S. Department of Health and Human Services, 2019) [55]); Table S1: Basal characteristics of the study population; Table S2: Mean differences for Verbal Fluency test according to Pb and Mn levels in biomarkers and PM personal sampling; Table S3: Mean differences for Trail Making Test (TMT) according to Pb and Mn levels in biomarkers and PM personal sampling; Table S4: Mean differences for Rey Osterrieth Complex Figure (ROCF) test according to Pb and Mn levels in biomarkers and PM personal sampling; Table S5: Mean differences for the Grooved Pegboard test (seconds) according to Pb and Mn levels in biomarkers and PM personal sampling in dominant and nondominant hands; Table S6: Mean differences for Finger Tapping Test (FTT) (number of taps in 10 s) according to Pb and Mn levels in biomarkers and PM personal sampling in dominant and nondominant hands.

Author Contributions: M.S. and I.F.-O.: conceptualization, methodology, writing—review and editing, supervision, and funding acquisition. L.R.-A. and B.M.: investigation and formal analysis. A.E.: investigation and resources. All authors have read and agreed to the published version of the manuscript.

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.

Institutional Review Board Statement: This study was conducted following the Declaration of Helsinki and approved by the Ethical Committee of Research of the University of Cantabria (CEUC) and the Ethical Committee of Clinical Research in Cantabria (CEIC) (protocol code 2017.164, date of approval 5 September 2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data cannot be made publicly available to protect patient privacy. The data are available on request from the University of Cantabria Archive (http://repositorio.

unican.es/ (accessed on 1 March 2024)) for researchers who meet the criteria for access to confidential data. Requests may be sent to the Ethics Committee (ceicc@idival.org) or Miguel Santibáñez (santibanezm@unican.es).

Acknowledgments: We would like to thank María Sierra, Isabel González-Aramburu, Ana Pozueta, 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.

Conflicts of 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.

References

1. European Commission (EC). Council Directive 2004/107/EC, Directive of the European Parliament and of the Council of 15 December 2004 relating to arsenic, cadmium, mercury, nickel and polycyclic aromatic hydrocarbons in ambient air. *Eur. Parliam. Counc. Eur. Union Off. J.* **2004**, *L23*, 3–16.
2. European Commission (EC). Council Directive 2008/50/EC, Directive of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe. *Eur. Parliam. Counc. Eur. Union Off. J.* **2008**, *L152*, 1–44.
3. U.S. EPA (Environmental Protection Agency). *Review of the National Ambient Air Quality Standards for Lead*; 40 CFR Part 50. Rules and Regulations. Federal Register; Environmental Protection Agency: Washington, DC, USA, 2016; Volume 81, pp. 71906–71943.
4. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Lead. Agency for Toxic Substances and Disease Registry*; U.S. Department of Health and Human Services: Washington, DC, USA, 2020.
5. Egan, K.B.; Cornwell, C.R.; Courtney, J.G.; Ettinger, A.S. Blood Lead Levels in U.S. Children Ages 1–11 Years, 1976–2016. *Environ. Health Perspect.* **2021**, *129*, 37003. [CrossRef]
6. Tsoi, M.-F.; Cheung, C.-L.; Cheung, T.T.; Cheung, B.M.Y. Continual Decrease in Blood Lead Level in Americans: United States National Health Nutrition and Examination Survey 1999–2014. *Am. J. Med.* **2016**, *129*, 1213–1218. [CrossRef] [PubMed]
7. Pirkle, J.L.; Brody, D.J.; Gunter, E.W.; Kramer, R.A.; Paschal, D.C.; Flegal, K.M.; Matte, T.D. The decline in blood lead levels in the United States. The National Health and Nutrition Examination Surveys (NHANES). *JAMA* **1994**, *272*, 284–291. [CrossRef]
8. Weuve, J.; Korrick, S.A.; Weisskopf, M.A.; Ryan, L.M.; Schwartz, J.; Nie, H.; Grodstein, F.; Hu, H. Cumulative exposure to lead in relation to cognitive function in older women. *Environ. Health Perspect.* **2009**, *117*, 574–580. [CrossRef]
9. Power, M.C.; Korrick, S.; Tchetgen, E.J.T.; Nie, L.H.; Grodstein, F.; Hu, H.; Weuve, J.; Schwartz, J.; Weisskopf, M.G. Lead exposure and rate of change in cognitive function in older women. *Environ. Res.* **2014**, *129*, 69–75. [CrossRef]
10. Weisskopf, M.G.; Proctor, S.P.; Wright, R.O.; Schwartz, J.; Spiro, A.I.; Sparrow, D.; Nie, H.; Hu, H. Cumulative lead exposure and cognitive performance among elderly men. *Epidemiology* **2007**, *18*, 59–66. [CrossRef]
11. Seo, J.; Lee, B.-K.; Jin, S.-U.; Park, J.W.; Kim, Y.-T.; Ryeom, H.-K.; Lee, J.; Suh, K.J.; Kim, S.H.; Park, S.-J.; et al. Lead-induced impairments in the neural processes related to working memory function. *PLoS ONE* **2014**, *9*, e105308. [CrossRef]
12. Bleecker, M.L.; Lindgren, K.N.; Ford, P.D. Differential contribution of current and cumulative indices of lead dose to neuropsychological performance by age. *Neurology* **1997**, *48*, 639–645. [CrossRef]
13. Ryan, C.M.; Morrow, L.; Parkinson, D.; Bromet, E. Low Level Lead Exposure and neuropsychological functioning in blue collar males. *Int. J. Neurosci.* **1987**, *36*, 29–39. [CrossRef]
14. Shih, R.A.; Glass, T.A.; Bandeen-Roche, K.; Carlson, M.C.; Bolla, K.I.; Todd, A.C.; Schwartz, B.S. Environmental lead exposure and cognitive function in community-dwelling older adults. *Neurology* **2006**, *67*, 1556–1562. [CrossRef]
15. Shih, R.A.; Hu, H.; Weisskopf, M.G.; Schwartz, B.S. Cumulative lead dose and cognitive function in adults: A review of studies that measured both blood lead and bone lead. *Environ. Health Perspect.* **2007**, *115*, 483–492. [CrossRef]
16. Grashow, R.; Spiro, A.; Taylor, K.M.; Newton, K.; Shrairman, R.; Landau, A.; Sparrow, D.; Hu, H.; Weisskopf, M. Cumulative lead exposure in community-dwelling adults and fine motor function: Comparing standard and novel tasks in the VA Normative Aging Study. *NeuroToxicology* **2013**, *35*, 154–161. [CrossRef]
17. Coetzee, D.J.; McGovern, P.M.; Rao, R.; Harnack, L.J.; Georgieff, M.K.; Stepanov, I. Measuring the impact of manganese exposure on children's neurodevelopment: Advances and research gaps in biomarker-based approaches. *Environ. Health* **2016**, *15*, 91. [CrossRef]
18. Leonhard, M.J.; Chang, E.T.; Loccisano, A.E.; Garry, M.R. A systematic literature review of epidemiologic studies of developmental manganese exposure and neurodevelopmental outcomes. *Toxicology* **2019**, *420*, 46–65. [CrossRef]
19. Chen, P.; Bornhorst, J.; Aschner, M. Manganese metabolism in humans. *Front. Biosci.* **2018**, *23*, 1655–1679. [CrossRef]
20. World Health Organization. *Air Quality Guidelines for Europe*; World Health Organization: Geneva, Switzerland, 2000.
21. U.S. EPA. *Health Assessment Document for Manganese*; Final Report; EPA/600/8-83/013F; Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office: Cincinnati, OH, USA, 1984.
22. Winder, B.S.; Salmon, A.G.; Marty, M.A. Inhalation of an essential metal: Development of reference exposure levels for manganese. *Regul. Toxicol. Pharmacol.* **2010**, *57*, 195–199. [CrossRef] [PubMed]
23. Hernández-Pellón, A.; Fernández-Olmo, I. Airborne concentration and deposition of trace metals and metalloids in an urban area downwind of a manganese alloy plant. *Atmos. Pollut. Res.* **2019**, *10*, 712–721. [CrossRef]

24. Otero-Pregigueiro, D.; Hernández-Pellón, A.; Borge, R.; Fernández-Olmo, I. Estimation of PM10-bound manganese concentration near a ferromanganese alloy plant by atmospheric dispersion modelling. *Sci. Total. Environ.* **2018**, *627*, 534–543. [CrossRef] [PubMed]
25. Expósito, A.; Markiv, B.; Ruiz-Azcona, L.; Santibáñez, M.; Fernández-Olmo, I. Personal inhalation exposure to manganese and other trace metals in an environmentally exposed population: Bioaccessibility in size-segregated particulate matter samples. *Atmos. Pollut. Res.* **2021**, *12*, 101123. [CrossRef]
26. Fulk, F.; Haynes, E.N.; Hilbert, T.J.; Brown, D.; Petersen, D.; Reponen, T. Comparison of stationary and personal air sampling with an air dispersion model for children's ambient exposure to manganese. *J. Expo. Sci. Environ. Epidemiol.* **2016**, *26*, 494–502. [CrossRef]
27. Mbengue, S.; Alleman, L.Y.; Flament, P. Bioaccessibility of trace elements in fine and ultrafine atmospheric particles in an industrial environment. *Environ. Geochem. Health* **2015**, *37*, 875–889. [CrossRef] [PubMed]
28. Kastury, F.; Smith, E.; Karna, R.R.; Scheckel, K.G.; Juhasz, A. Methodological factors influencing inhalation bioaccessibility of metal(loid)s in PM2.5 using simulated lung fluid. *Environ. Pollut.* **2018**, *241*, 930–937. [CrossRef] [PubMed]
29. Weggeberg, H.; Benden, T.F.; Lierhagen, S.; Steinnes, E.; Flaten, T.P. Characterization and bioaccessibility assessment of elements in urban aerosols by extraction with simulated lung fluids. *Environ. Chem. Ecotoxicol.* **2019**, *1*, 49–60. [CrossRef]
30. Ruiz-Azcona, L.; Markiv, B.; Expósito, A.; González-Aramburu, I.; Sierra, M.; Fernández-Olmo, I.; Santibáñez, M. Biomonitoring and bioaccessibility of environmental airborne manganese in relation to motor function in a healthy adult population. *NeuroToxicology* **2021**, *87*, 195–207. [CrossRef] [PubMed]
31. Ruiz-Azcona, L.; Markiv, B.; Expósito, A.; Pozueta, A.; García-Martínez, M.; Fernández-Olmo, I.; Santibáñez, M. Poorer cognitive function and environmental airborne Mn exposure determined by biomonitoring and personal environmental monitors in a healthy adult population. *Sci. Total. Environ.* **2022**, *815*, 152940. [CrossRef] [PubMed]
32. Golden, C.J. *STROOP: Test de Colores y Palabras*, 3rd ed.; TEA Ediciones, S.A.: Madrid, Spain, 2001.
33. Wechsler, D. *Escala de Inteligencia de Weschler para Adultos III. Manual de Aplicación y Corrección*, 2nd ed.; TEA Ediciones: Madrid, Spain, 2001; pp. 149–153.
34. Ruff, R.; Light, R.; Parker, S.; Levin, H. The psychological construct of word fluency. *Brain Lang.* **1997**, *57*, 394–405. [CrossRef] [PubMed]
35. Partington, J.; Leiter, R. Partington's pathways test. *Psychol. Serv. Cent. Bull.* **1949**, *1*, 9–20.
36. Reitan, R.M.; Wolfson, D. The Halstead-Reitan neuropsychological test battery. In *Theory and Clinical Interpretation*, 2nd ed.; Neuropsychology Press: Tucson, AZ, USA, 1993.
37. Rey, A. *Manual Rey: Test de Copia y de Reproducción de Memoria de Figuras Geométricas Complejas*, 8th ed.; TEA Ediciones: Madrid, Spain, 2003.
38. Peña-Casanova, J.; Blesa, R.; Aguilar, M.; Gramunt-Fombuena, N.; Gómez-Ansón, B.; Oliva, R.; Molinuevo, J.L.; Robles, A.; Barquero, M.S.; Antúnez, C.; et al. Spanish Multicenter Normative Studies (NEURONORMA Project): Methods and Sample Characteristics. *Arch. Clin. Neuropsychol.* **2009**, *24*, 307–319. [CrossRef]
39. Lezak, M.D. *Neuropsychological Assessment*, 4th ed.; Oxford University Press: New York, NY, USA, 2004.
40. Bornstein, R.A. Normative data on intermanual differences on three tests of motor performance. *J. Clin. Exp. Neuropsychol.* **1986**, *8*, 12–20. [CrossRef]
41. Oteo, J.A.; Benavente, P.; Garzón, M. Securities regulatory force fist in Spanish working age population. Anthropometric influence of variables of the hand and forearm [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] [Spanish]. *Rev. Iberoam. Cir. Mano* **2015**, *43*, 104–110. [CrossRef]
42. Eastman, R.R.; Jursa, T.P.; Benedetti, C.; Lucchini, R.G.; Smith, D.R. Hair as a biomarker of environmental manganese exposure. *Environ. Sci. Technol.* **2013**, *47*, 1629–1637. [CrossRef] [PubMed]
43. Markiv, B.; Ruiz-Azcona, L.; Expósito, A.; Santibáñez, M.; Fernández-Olmo, I. Short- and long-term exposure to trace metal(loid)s from the production of ferromanganese alloys by personal sampling and biomarkers. *Environ. Geochem. Health* **2022**, *44*, 4595–4618. [CrossRef] [PubMed]
44. Graney, J.R.; Landis, M.S.; A Norris, G. Concentrations and solubility of metals from indoor and personal exposure PM2.5 samples. *Atmos. Environ.* **2004**, *38*, 237–247. [CrossRef]
45. Pollitt, K.J.G.; Maikawa, C.L.; Wheeler, A.J.; Weichenthal, S.; Dobbin, N.A.; Liu, L.; Goldberg, M.S. Trace metal exposure is associated with increased exhaled nitric oxide in asthmatic children. *Environ. Health* **2016**, *15*, 94. [CrossRef]
46. Lucchini, R.G.; Guazzetti, S.; Zoni, S.; Donna, F.; Peter, S.; Zacco, A.; Salmistraro, M.; Bontempi, E.; Zimmerman, N.J.; Smith, D.R. Tremor, olfactory and motor changes in Italian adolescents exposed to historical ferro-manganese emission. *NeuroToxicology* **2012**, *33*, 687–696. [CrossRef]
47. Haynes, E.N.; Ryan, P.; Chen, A.; Brown, D.; Roda, S.; Kuhnell, P.; Wittberg, D.; Terrell, M.; Reponen, T. Assessment of personal exposure to manganese in children living near a ferromanganese refinery. *Sci. Total. Environ.* **2012**, *427–428*, 19–25. [CrossRef]
48. Solís-Vivanco, R.; Rodríguez-Agudelo, Y.; Riojas-Rodríguez, H.; Ríos, C.; Rosas, I.; Montes, S. Cognitive impairment in an adult Mexican population non-occupationally exposed to manganese. *Environ. Toxicol. Pharmacol.* **2009**, *28*, 172–178. [CrossRef]
49. Strauss, E.; Sherman, E.M.S.; Spreen, O. *A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary*, 3rd ed.; Oxford University Press: New York, NY, USA, 2006.

50. Casjens, S.; Pesch, B.; van Thriel, C.; Zschiesche, W.; Behrens, T.; Weiss, T.; Pallapies, D.; Arendt, M.; Dragano, N.; Moebus, S.; et al. Associations between blood lead, olfaction and fine-motor skills in elderly men: Results from the Heinz Nixdorf Recall Study. *NeuroToxicology* **2018**, *68*, 66–72. [CrossRef]
51. Baker, E.L.; Feldman, R.G.; White, R.F.; Harley, J.P. The role of occupational lead exposure in the genesis of psychiatric and behavioral disturbances. *Acta Psychiatr. Scand.* **1983**, *67*, 38–48. [CrossRef]
52. Hanninen, H.; Aitio, A.; Kovala, T.; Luukkonen, R.; Matikainen, E.; Mannelin, T.; Erkkila, J.; Riihimaki, V. Occupational exposure to lead and neuropsychological dysfunction. *Occup. Environ. Med.* **1998**, *55*, 202–209. [CrossRef] [PubMed]
53. A Maizlish, N.; Parra, G.; Feo, O. Neurobehavioural evaluation of Venezuelan workers exposed to inorganic lead. *Occup. Environ. Med.* **1995**, *52*, 408–414. [CrossRef] [PubMed]
54. Schwartz, B.S.; Lee, B.-K.; Lee, G.-S.; Stewart, W.F.; Lee, S.-S.; Hwang, K.-Y.; Ahn, K.-D.; Kim, Y.-B.; Bolla, K.I.; Simon, D.; et al. Associations of blood lead, dimercaptosuccinic acid-chelatable lead, and tibia lead with neurobehavioral test scores in South Korean lead workers. *Am. J. Epidemiol.* **2001**, *153*, 453–464. [CrossRef] [PubMed]
55. U.S. Department of Health and Human Services; Centers for Disease Control and Prevention. *National Center for Environmental Health. Division of Laboratory Sciences. Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables*; National Center for Environmental Health: Washington, DC, USA, 2019; Volume 1.