Tremor, olfactory and motor changes in Italian adolescents exposed to historical ferro-manganese emission

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ABSTRACT

Background and objective: Increased prevalence of Parkinsonism was observed in Valcamonica, Italy, a region impacted by ferroalloy plants emissions containing manganese and other metals for a century until 2001. The aim of this study was to assess neurobehavioral functions in adolescents from the impacted region and the reference area of Garda Lake.

Methods: Adolescents age 11–14 years were recruited through the school system for neuro-behavioral testing. Metals including manganese, lead, iron, zinc, copper were measured in airborne particulate matter collected with 24-h personal samplers, and in soil, tap water, blood, urine and hair. Independent variables included parental education and socio-economic status, children's body mass index, number of siblings, parity order, smoking and drinking habits.

Results: A total of 311 subjects (49.2% females), residing in either the exposed (n = 154) or the reference (n = 157) area participated. Average airborne and soil manganese were respectively 49.5 ng/m³ (median 31.4, range 1.24–517) and 958 ppm (median 897, range 465–1729) in the impacted area, and 27.4 ng/m³ (median 24.7, range 53–85.9) and 427 ppm (median 409 range 160–734) in the reference area. Regression models showed significant impairment of motor coordination (Luria-Nebraska test, p = 0.0005), hand dexterity (Aiming Pursuit test, p = 0.0115) and odor identification (Sniffin' task, p = 0.003) associated with soil manganese. Tremor intensity was positively associated with blood (p = 0.005) and hair (p = 0.01) manganese.

Conclusion: Historical environmental exposure to manganese from ferroalloy emission reflected by the concentration in soil and the biomarkers was associated with sub-clinical deficits in olfactory and motor function among adolescents.

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1. Introduction

Early life exposure to metals such as lead (Pb) and manganese (Mn) has been shown to cause neurotoxic effects of particular concern in susceptible subgroups like children. While pediatric exposure to Pb has been extensively studied for cognitive impairment, the neuro-developmental effect of Mn has been ascertainment only recently. Exposure to Mn in water (Wasserman et al., 2006; Bouchard et al., 2011) and airborne dust (Riojas-Rodriguez et al., 2010; Menezes-Filho et al., 2011) has been found to be associated with cognitive impairment measured as reduced IQ. Since Mn neurotoxicity is known for extrapyramidal effects in adults and has been related to early Parkinsonism (Lucchini et al., 2007), control of motor function may be impaired also in younger individuals after early life exposure. Exposure to Mn can occur from various sources via inhalation and ingestion routes. Recent animal studies have also suggested that the olfactory uptake of airborne particulates may be important in brain Mn uptake, since it would circumvent physiological barriers (e.g., blood–brain barrier) that normally regulate to some extent brain uptake (Aschner and Dorman, 2006). Although studies on the uptake of Mn through the human olfactory system are not available, it is likely that olfactory transport of Mn plays an important role in human neurotoxicity (Lucchini et al., 2010).
2.1. Study sites

The study area includes different sites of the Province of Brescia, Northern Italy (Fig. 1): Valcamonica, a valley of the pre-Alps that runs for about 50 miles in the NE-SW direction with an average width of about 2 miles, and is delimited by mountains of about 10,000 feet. Winds in the valley average 3 miles/h primarily in the SW → NE direction during the day and NE → SW during the night, with no seasonal variation. Here three ferroalloy plants have been operating in the municipalities of the province of Brescia (Kruskai–Wallis chi-squared 1 df = 17.55, p-value <0.001). The SMRs were positively associated with Mn levels in outdoor deposited dust (Lucchini et al., 2007).

In light of these findings, we undertook a cross sectional study on behavior, cognitive and motor functions among healthy individuals resident in Valcamonica and in a reference non industrial area of the province of Brescia, located on the west shore of the Garda Lake, where concentrations of metals in deposited dust are significantly lower (Zacco et al., 2009). Different age groups were targeted by this project, including pregnant mothers and infants, adolescents, ferroalloy workers, elderly. Here we report the results on the assessment of motor and neurosensory functions among the adolescents; the investigation of cognitive and behavioral functions and in the other age-groups will be published elsewhere.

2. Methods

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2.2. Participant enrollment

Children were enrolled through the public school system according to a community-based participatory approach that involved the local communities of Valcamonica and Garda Lake. The aims and methodology of the study were explained through community meetings and conferences and publicized by the local media. Teachers, parents and children were informed with ad hoc meetings and brochures. Subjects who agreed to participate filled in a screening questionnaire for the evaluation of inclusion and exclusion criteria. The inclusion criteria included: (i) to be born in the study area from resident families living in the study area since the 1970s; (ii) to live in the study area since birth; (iii) to be aged 11–14 years. The exclusion criteria were represented by: (i) pathological conditions potentially affecting neuro-development, including neurological, hepatic, metabolic, endocrine and psychiatric diseases; (ii) consumption of medications with known neuro-psychological side-effects; (iii) clinically diagnosed motor deficits of hand and fingers; (iv) clinically diagnosed cognitive impairment and behavioral manifestations; (v) visual deficits not adequately corrected. General practitioners physicians and pediatricians of the study areas were informed about the research aims and methods. The study design, the information about the study aims and the forms for informed consent had been reviewed and approved by the ethical committees of the local Public Health agencies of Valcamonica and Brescia.

2.3. Study design

The assessment of participant subjects was divided in three sessions, and carried out on different days over 2 consecutive weeks. Trained medical doctors and neuro-psychologists conducted the first session within dedicated rooms in the local school.
The third session was dedicated to the collection of biological Anthropometric data were measured for the calculation of body area at each site; the session included the collection of socio­exposure data and hematological parameters were communicated exposure, including blood, urine, and hair. Blood samples were also available exposure protective standards and the normative data for an anonymized identification codes accessible only to the research team, and under the responsibility of the study Principal Investigator (RL). According to an ethical procedure on open information about the final study outcomes, individual results on the return of X-ray fluorescence (TXRF) spectroscopy, according to a methodology published elsewhere (Borgese et al., 2011, 2012). Water was sampled from the primary use home tap after a 2 min run at a medium flow rate and examined with TXRF multi­elemental analysis, that has a lowest detection limit for Mn in water of 1 µg/L. The metal content in the soil was analyzed in situ with a portable X-ray fluorescence (XRF) instrument (Thermo Scientific Niton, model XL3t) equipped with GPS geo­referencing capability. The Niton portable XRF analyzes soil for metals by the generation of an X-ray signal and the instrument interpretation of the energies of the returning X-ray fluorescence signal from the excitation of the metal elements in the soil. Sample collection times ranged from 60 to 100 s. Surface soil measurements were collected in both the impacted and the reference areas as well as in the yards of a select number of subjects' homes. At the homes, two to four randomly spaced readings were taken and averaged. The instrument was internally calibrated prior to each usage. In addition, a series of soil standards reference materials (NIST 2780, 2709a) produced by the U.S. National Institute of Standards and Technology (NIST) were measured at the beginning of each sampling session with the Niton XRF. The collected filters for airborne particles were analyzed in accordance with the patented method PCT/IT2008/000458 (Depero et al., 2009) which allows the filter to be analyzed directly and non-destructively without any chemical treatment using total X-ray fluorescence (TXRF) techniques. The TXRF measurements were performed with the Bruker TXRF system S2 Picofox, air cooled, Mo tube, Silicon­Drift Detector, with operating values of 50 kV and 1000 µA, using an acquisition time of 600 s. The absolute concentration of the elements in the filters was evaluated through calibration with an air particulate standard filter (NIST CRM 2783).

A Food Frequency Questionnaire (FFQ) was used to estimate the daily dietary intake of Mn. The questionnaire included specific food items such as cereals, whole grain products, vegetable (chard, spinach), beans, lentils, chickpeas, fruit (pineapple, hazelnut), tea, cacao, coffee, of known higher Mn content based on the US nutrient database (USDA, 2010). The intake
was portion-adjusted based on Italian reference data on food consumption (Leclercq et al., 2009).

2.5. Collection and analysis of exposure biomarkers

Exposure to Mn and Pb was also measured in total blood and urine. Venous whole blood samples (4 mL) were collected using a 19-gauge butterfly catheter into a Li-Heparin Sarstedt Monovette Vacutainer. A spot urine sample (50–200 mL) was collected into a clean, sterile polyethylene container. All samples were stored at 4 °C until analyzed at the laboratory facility of the University of Brescia. Hematological tests were performed to assess iron metabolism and liver function. Hair samples were collected from each subject using stainless steel scissors. Briefly, a 2–3 cm section of hair proximal to the scalp (~100–150 strands, or ~20 mg) was collected from the nape of the neck and stored in polyethylene bags at room temperature until analyses.

Manganese and Pb measurements in blood and Mn in urine (MnU) were performed by Zeeman graphite furnace atomic absorption spectrometry (Varian SpectrAA) in the industrial hygiene laboratory at the University of Brescia, Italy, using methods previously reported (Apostoli et al., 2000). Levels of Mn in hair (MnH) were determined by magnetic sector inductively coupled plasma mass spectrometry (Thermo XR-Thermo Element XR ICP-MS). Briefly, hair samples (~10–50 mg) were cleaned of exogenous metal contamination as follows: sonication (20 min) in 0.5% Triton, rinsing 5× with Milli-Q ultrapure water, sonication (10 min) in 1 N trace metal grade nitric acid, rinse with 1 N nitric acid, and rinsing 3× with Milli-Q water. Clean hair samples were dried overnight at 60 °C in a HEPA filtered-air clean room.

Subsequently, hair was transferred to pre-weighed 6 mL poly tubes and digested in 0.5 mL 15.7 N double quartz-distilled nitric acid at 80 °C for 6 h in a Class-100 HEPA filtered-air hood. Following digestion, 5 mL Milli-Q water was added to each tube, and tubes were capped and vortexed. Rhodium and thallium were added as internal standards, and samples were analyzed for Pb-208 and Th-205 (low resolution), and Cr-52, Mn-55, Cu-63, and Rh-103 (medium resolution). Metal concentrations were determined by comparison with certified multi-element standards (Spec Industries). The analytical detection limits for Mn, Pb, Cu, and Cr were 0.0054, 0.0027, 0.027, and 0.0019 ng/mL, respectively, based on repeated measurements of procedural blanks on four different analysis days.

2.6. Neuro-psychological testing

The neurobehavioral test battery was designed based on a review of the tests reported in the Mn literature (Zoni et al., 2007). Since pre-clinical early signs of neurotoxicity were considered in the study design, sensitive neurobehavioral tests were selected to capture exposure-related changes also in the normality range. Based on the study design and the exclusion criteria adopted, normative data were not used in the analysis.

Similarly to other studies on manganese neurotoxicity (Zoni et al., 2007) motor coordination was explored with 5 subtests of the Luria Nebraska Motor Battery (Golden et al., 1980), a standardized test battery composed by a total of 11 clinical scales. The test we administered, each lasting 10 s, were: dominant hand clenched, non-dominant hand clenched, alternative hand clenched, finger-thumb touching with dominant hand, and finger-thumb touching with non-dominant hand. The sum of the scores of these 5 subtests yielded a final score that represents, therefore, a partial Luria motor score.

For motor coordination we used also a computerized version of the Finger Tapping test, computerized version from the SPES (Iregren et al., 1996): task of participant is to tap a push-button alternatively with the dominant and non-dominant hand within 5 min. From the same computerized system we selected also the Visual Simple Reaction time test for vigilance and psychomotor response: the reaction time is the time required to respond to the presence of a visual stimulus represented by a red rectangle appearing on the computer screen. The subject is asked to press a button as soon as a stimulus appears on the screen. Hand dexterity and perceptual speed were assessed with the Pursuit Aiming test (Fleischman, 1954). For this test, the subject must place a dot with a pencil inside 2 mm diameter circles as quickly as possible for two series of 60 s each. Tremor was assessed by the Tremor 7.0 of Danish Products Development- DP (Després et al., 2000). During the test, the subject holds a stylus for 10 s and the hand vibrations are recorded and displayed in a time axis plot. The accelerations are analysed by methods drawn from vibrations measurements. Body sway was recorded by a balance plate (SWAY system by DPD), which produces signals from three sensors to provide a map of the position of the force center during the test period. This centre is defined as the center of equilibrium of the three vertical forces, recorded at the three supports of the sway plate. During the test the subject stands erect on the sway plate and the change in position of the force center can be observed in an X–Y coordinate system. The test was repeated in two conditions of open and closed eyes. To assess odor identification we used Sniffin' Sticks-Offactory Screening 12 test (Burghart Medical Technology). The subject smells the tips of 12 fiber sticks filled with different common scents and must identify the odors from a list of 4 concepts each. The result of the test is the sum of the correctly identified odors. We selected this test because the smells are more familiar to the Italian subjects compared to other produced elsewhere, and also because this test was already successfully used in children (Hummel et al., 2007).

2.7. Statistical analysis

Preliminary non-parametric statistics (Mann-Whitney and Kruskal-Wallis) were used to test the null hypothesis that the observed values were drawn from the same population in the impacted and reference area. Since air samples and soil measurements were not collected for the entire population we used GIS Kriging interpolations to obtain values for the missing points and gain power in the subsequent regression analyses. The median distance between points with and without direct soil measurements was 780 m (20 m–5.37 km), while for the air measures the median distance was 119 m (range 20 m–1.9 km). Ordinary Kriging is a geostatistical technique used to estimate data at unobserved locations from the observations at nearby locations and is based on spatial correlation of the data. Therefore we used a Stratified (by area) Ordinary Kriging model based on log-transformed Mn levels to estimate the unobserved individual soil Mn values. Since spatial correlation of airborne measurements was weak, we used Inverse Weighted Distance interpolation (Bivand et al., 2008) to estimate the unobserved air Mn values. Cross-validation was used to assess the reliability of the Kriging models. To test the hypothesis that Mn exposure affects motor and sensory functions in children and to obtain an adjusted estimate of the effect we used multiple (linear and logistic) regression analysis. Deviations from linearity in the relationship between exposure measures and covariate and dependent variables was evaluated comparing the ordinary least square model (OLS) or the logistic model with a semi-parametric generalized additive model (GAM) where the choice of the smoothness of the non-parametric part of the model was driven by generalized cross validation (Wood, 2006). When linearity could not be rejected we reduced the model to the simplest OLS (or logistic) model or we entered the continuous variable parametrically in the GAM model. We used soil Mn levels as the surrogate of Mn exposure to the subjects because of the collinearity (concurvity).
between the air and soil Mn levels. Using only the soil Mn data resulted in more stable models and no gain in information was obtained when retaining the air Mn data in the models, as indicated by Akaike's information criterion (Venables and Ripley, 2002). We retained in the regression models all the variables that could have been related both to the exposure and to the outcome, including age, gender, SES, family size, parity order, maternal and paternal education, alcohol and smoking habits, BMI. These variable were included on an "a priori" basis, even when they showed to be statistically non significant, in order to obtain an unbiased estimate of the effect of Mn exposure (Harrell, 2001). All the analyses and graphics were made with R (R Development Core Team, 2011).

3. Results

3.1. Social-demographics

The study included 311 adolescents from different families and households, selected from a total of 414 originally enrolled. Exclusion was due to: not meeting the residence criteria (n = 61); refusal to participate (n = 22), neurological and psychiatric diseases (n = 12); incomplete informed consent (n = 5); endocrine diseases (n = 1); metabolic disease (n = 1); total parental nutrition (n = 1). Participants resided in Valcamonica (n = 154) and in the Garda Lake area (n = 157). According to the three sites of previous ferroalloy operations, the subjects from Valcamonica were further sub-classified in three areas including upper- (n = 23), mid- (n = 85), lower- (n = 46) Valcamonica.

Gender was equally distributed between females (49.2%) and males (50.8%), and median age was 155 months, with 18.3% of 11 years, 33.8% of 12 years, 40.5% of 13 years, 7.4% of 14 years old subjects. SES was low in 24.8%, medium in 41.2% and high in 34% of the families, and maternal education low in 38%, medium in 52.3% and high in 9.7% of the cases. Participants with no siblings were 15.3%, one sibling 61.7%, two siblings 19.5%, three or more 3.6%. Parity order was first in 52%, second in 38.3% and third or more with a comparison between the Valcamonica and Garda Lake areas, although a tendency toward increased levels from the lower to mid-upper valley was noticed for all biomarkers. The mean levels of Pb in blood were extremely low in subjects in both study areas: Valcamonica mean 1.72 µg/dL (median 1.60), Garda Lake 1.60 µg/dL (median 1.40). The concentration of Mn in airborne particles was higher in Valcamonica, especially in the mid valley (median 51.73 ng/m³), compared to the Garda Lake reference area (median 24.72 ng/m³). Concentrations of the other metals indicated a similar trend for Pb, Cr, Fe, Ni, whereas Cu and Zn were higher in the Garda Lake, possibly due to the agricultural use of chemical products (data not shown). Airborne PM10 particles were also slightly higher in mid Valcamonica (mean 71.64, median 62.2 µg/m³) compared to the Garda Lake (mean 63.76, median 58.93 µg/m³). The subjects' diaries kept over the 24 h air sampling period showed an average of 2 h outdoor and 22 h indoor, with slight seasonal variation (1 h outdoor in winter time and 3–5 h in spring time), and no variation across the different study areas. Data on rain precipitation showed that 46% of air particles were collected over 24 h periods that contained some rain, while 54% were collected with no rain during the sampling time, with slight differences between the Valcamonica (40% rain and 60% no rain) and Garda Lake (58% rain and 42% no rain) areas.

Soil values showed a significant increase from the lowest concentration in Garda Lake to the lower-, mid-, upper-Valcamonica sub-areas for Mn and for Pb, Fe, Zn, As, Ni (data not shown).

The concentrations of heavy metals in tap water, reflecting the public water supply from either study area, were not elevated. In fact, water Mn levels were below the LDL of 1 µg/L in all water samples. Only two subjects from Valcamonica (1.30% of participants) and two subjects from the Garda Lake (1.27% of participants) reported drinking water from private wells. The estimated daily oral intake of Mn based on dietary questionnaire was 2.91 ± 1.5 mg/day (median 2.66, range 0.72–9.46) for all participants, with no area differences.

Data on Mn exposure assessment are summarized in Table 1a with a comparison between the Valcamonica and Garda Lake areas, and in Table 1b with sub-grouping of Valcamonica according to the former location of the three ferroalloy plants. The average values for the entire Valcamonica were: MnH 0.16 µg/g (median 0.11), MnB 10.99 µg/L (median 10.8), MnU 0.22 µg/L (median 0.10), Mn Air 49.47 ng/m³ (median 31.39), Mn Soil 958.16 ppm (median 897.49). No significant differences were noted for the biomarkers MnH, MnB, and MnU between Valcamonica and Garda Lake, although a tendency toward increased levels from the lower to mid-upper valley was noticed for all biomarkers. The mean levels of Pb in blood were extremely low in subjects in both study areas: Valcamonica mean 1.72 µg/dL (median 1.60), Garda Lake 1.60 µg/dL (median 1.40). The concentration of Mn in airborne particles was higher in Valcamonica, especially in the mid valley (median 51.73 ng/m³), compared to the Garda Lake reference area (median 24.72 ng/m³). Concentrations of the other metals indicated a similar trend for Pb, Cr, Fe, Ni, whereas Cu and Zn were higher in the Garda Lake, possibly due to the agricultural use of chemical products (data not shown). Airborne PM10 particles were also slightly higher in mid Valcamonica (mean 71.64, median 62.2 µg/m³) compared to the Garda Lake (mean 63.76, median 58.93 µg/m³). The subjects' diaries kept over the 24 h air sampling period showed an average of 2 h outdoor and 22 h indoor, with slight seasonal variation (1 h outdoor in winter time and 3–5 h in spring time), and no variation across the different study areas. Data on rain precipitation showed that 46% of air particles were collected over 24 h periods that contained some rain, while 54% were collected with no rain during the sampling time, with slight differences between the Valcamonica (40% rain and 60% no rain) and Garda Lake (58% rain and 42% no rain) areas.

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### Table 1a
Manganese exposure assessment in biomarkers and environmental media (VC, Valcamonica; GL, Garda Lake).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Levels</th>
<th>n</th>
<th>Min</th>
<th>1st q.LE</th>
<th>Mean</th>
<th>Median</th>
<th>3rd q.LE</th>
<th>Max</th>
<th>IQR</th>
<th>θNA</th>
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</thead>
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<td>Mn hair (ppm)</td>
<td>VC</td>
<td>125</td>
<td>0.02</td>
<td>0.07</td>
<td>0.16</td>
<td>0.11</td>
<td>0.19</td>
<td>1.27</td>
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<td></td>
<td>GL</td>
<td>133</td>
<td>0.02</td>
<td>0.06</td>
<td>0.18</td>
<td>0.12</td>
<td>0.20</td>
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<tr>
<td>Mn blood (µg/L)</td>
<td>VC</td>
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<td>4.00</td>
<td>8.70</td>
<td>10.99</td>
<td>10.80</td>
<td>13.15</td>
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<tr>
<td></td>
<td>GL</td>
<td>148</td>
<td>6.00</td>
<td>8.90</td>
<td>11.24</td>
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<tr>
<td>Mn urine (µg/L)</td>
<td>VC</td>
<td>151</td>
<td>0.10</td>
<td>0.10</td>
<td>0.22</td>
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<td>0.10</td>
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<td>0.10</td>
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<tr>
<td>Mn air (µg/m³)</td>
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3.3. Neuro-psychological testing

Differences between Valcmonica and Garda Lake subjects were noted for health outcomes including Sniffin' sticks task (p = 0.005), Aiming Pursuit test (p = 0.01), the Luria-Nebraska partial motor scale (p = 0.002), and body sway with closed eyes (p = 0.05) (Table 2). The multivariate analysis with GAM and (Generalized Linear Model-GLM) logistic models showed a significant association of soil Mn levels with the Luria-Nebraska motor coordination test (Table 3, Fig. 3), with the Aiming Pursuit hand steadiness test (Table 4, Fig. 4) and with Sniffin' sticks odor identification task (Table 5, Fig. 5). The smooth curves are represented using penalized regression splines with smoothing parameters selected by Generalized Cross Validation (Wood, 2006). A significant association was also observed between MnH and MnB and tremor intensity in the dominant hand, with a border-line relation also between soil Mn and tremor intensity.
Table 4
Adjusted effect of soil Mn on the Aiming Pursuit hand dexterity test score, semi-parametric (GAM) model.

| Estimate | SE   | z-Value | p(>|z|) |
|----------|------|---------|---------|
| Intercept| 129.7736 | 4.9641 | 26.142 | 0.0000 |
| Gender (M vs F) | -4.8431 | 3.0155 | -1.606 | 0.1094 |
| SES* | 1.1482 | 4.1185 | 0.279 | 0.7806 |
| SESb | 5.4496 | 4.9228 | 1.107 | 0.2692 |
| Maternal education* | -7.2377 | 3.6392 | -1.989 | 0.0477 |
| Maternal educationb | -4.1177 | 6.4537 | -0.638 | 0.5240 |
| Smoking habit | -22.4593 | 15.4284 | -1.456 | 0.1466 |
| Alcohol consumption | -4.5436 | 9.1090 | -0.499 | 0.6183 |

Non-parametric Approximate significance of smooth terms

<table>
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<th>Ref. Edf</th>
<th>F</th>
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<td>Mn soil</td>
<td>4.324</td>
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<td>2.922</td>
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</table>

*a Medium vs low.
*b High vs low.

Table 5. Although declared by a small number of subjects, alcohol consumption was negatively associated to odor identification (Table 5) and cigarette smoking increased tremor intensity (Table 6).

Table 5
Adjusted effect of soil Mn on the olfactory test, semi-parametric (GAM) logistic model.

| Estimate | SE   | z-Value | p(>|z|) |
|----------|------|---------|---------|
| Intercept | 2.6179 | 0.2363 | -6.079 | 0.0000 |
| Gender (M vs F) | 0.2564 | 0.0413 | 6.213 | <0.0001 |
| Smoking habit | 0.4172 | 0.1886 | 2.213 | 0.0279 |
| Alcohol consumption | -0.0153 | 0.1130 | -0.135 | 0.8924 |

Non-parametric Approximate significance of smooth terms

<table>
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<tbody>
<tr>
<td>Age</td>
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<td>4.623</td>
<td>22.354</td>
</tr>
</tbody>
</table>

*a Medium vs low.
*b High vs low.

Table 6
Adjusted effect of various Mn sources on (log-transformed) tremor intensity, semiparametric (GAM) model.

| Estimate | SE   | z-Value | p(>|z|) |
|----------|------|---------|---------|
| Intercept | -2.9765 | 0.0346 | -66.817 | 0.0000 |
| Gender (M vs F) | 0.2564 | 0.0413 | 6.213 | <0.0001 |
| Smoking habit | 0.4172 | 0.1886 | 2.213 | 0.0279 |
| Alcohol consumption | -0.0153 | 0.1130 | -0.135 | 0.8924 |

Non-parametric Approximate significance of smooth terms

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<td>Mn blood</td>
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*a Medium vs low.
*b High vs low.
4. Discussion

The present study shows an association between Mn exposure reflected by Mn levels in surface soil, hair and blood and impairment of motor coordination, hand dexterity, tremor intensity and odor identification in adolescents living in Valcamonica, Italy. Although Mn is a recognized extrapyramidal neurotoxicant in adults since the past century, the most recent studies on exposed children have focused only on cognitive and behavioral implications. Our work is the first showing clear Mn-related motor dysfunction in children, that are very similar to those already observed by our group in chronically exposed ferroalloy workers (Lucchini et al., 1995, 1999). Furthermore, this observation is of particular interest given that in the same region we observed a significantly increased prevalence of Parkinsonism in children from Quebec, where Mn levels averaged 795 μg/L (Wasserman et al., 2006). Water from the public supply system in the province of Brescia is treated to decrease Mn levels to the current guideline of 400 μg/L set by the World Health Organization and the U.S. health reference level of 300 μg/L (Ljung and Vahter, 2007). They are also lower than drinking water Mn levels ranging from 0.1 to 2700 μg/L (median 30.8 μg/L) from Toronto and Indianapolis. Background average levels of 10 ng/m³ have also been recently surveyed in urban areas in Spain, rising to 25 ng/m³ in industrial sites (Moreno et al., 2011). The current reference concentration for airborne Mn of USEPA and Health Canada in Canada is 50 ng/m³ (Health Canada, 2010). The soil Mn levels were relatively consistent in showing an increasing gradient from the lower to the upper Valcamonica, which may be explained by the day time prevailing wind direction (South to North) and also by an overlap of anthropogenic emission on naturally higher soil Mn levels in the upper Valcamonica. This was shown by previous fingerprint analysis of metals in the deposited dust sampled in the entire province of Brescia (Zacco et al., 2009). Further, differences regarding the ferroalloy plants previously operating in the three sub-areas of Valcamonica can partially explain the differences of metal concentrations in airborne particles and surface soil in these sub-areas. The lower Valcamonica shows air levels of metals and PM10 that are similar to the Garda Lake, probably because the plant in this area was closed in 1995, which is more than 10 years before this study. The mid-valley shows the highest air levels probably because the plant in this area was closed most recently in 2001 and because particle re-suspension and residual industrial emissions are still present in this area. The ferroalloy plant in the upper valley was mainly a holding/deposit site and for Mn-rich ores shows the highest soil Mn levels.

In this study, the very low levels of Mn in tap water indicate drinking water does not represent a notable source of exposure. The observed levels below 1 μg/L are substantially lower than the current guideline of 400 μg/L set by the World Health Organization and the U.S. health reference level of 300 μg/L (Ljung and Vahter, 2007). They are also lower than drinking water Mn levels associated with cognitive deficits in children from Québec, where water Mn levels ranged from 0.1 to 2700 μg/L (median 30.8) (Bouchard et al., 2011), and Bangladesh, where water Mn levels averaged 795 ± 755 μg/L (Wasserman et al., 2006). Water from the public supply system in the province of Brescia is treated to decrease the Mn level for esthetic reasons. This treatment is generally lacking in private wells, where Mn concentrations may reach high levels (Ljung and Vahter, 2007), but the use of water from private wells was very limited in the present study. Mn oral intake resulted in 81% (118 girls and 130 boys) above the Adequate Intake (AI) by the Institute of Medicine, for children 9–13 years of age (1.6 mg/d for girls; 1.9 mg/d for boys) and in 5% above the Upper Intake Level (UL) of 6 mg/d, although within the range of data reported in the literature (Ljung and
Vahter, 2007). Levels of Mn in blood, urine, or hair were not measurably different in subjects from the impacted Valcamonica versus Garda Lake reference areas, although MnB, MnU and MnH were slightly higher in the subjects from the mid-upper Valcamonica compared to the other two areas (lower Valcamonica or Garda Lake). Blood levels of Mn were similar to those recently measured in 359 school children aged 8–16 years from Durban, South Africa (10.1 ± 3.4 μg/l, median 9.6), exposed to similar Mn levels in PM10 airborne particles (48.7 ± 44 ng/m³, median 34.3) (Batterman et al., 2011). This is the first study that shows Mn-associated with extra-pyramidal impairment. Hair Mn levels have been associated with cognitive deficits in other studies (Wright et al., 2006; Bouchard et al., 2011; Rojas-Rodríguez et al., 2010; Menezes-Filho et al., 2011), though the extent that hair Mn levels reported in those studies reflects external contamination versus internal body burden is not clear, since the hair cleaning methodologies varied widely in those studies, and all of them were notably less rigorous than the methods used in the present study.

A limitation of this study may be the possible residual confounding by unobserved variables. The study is based on a comparison between two adolescents populations that we assumed to be similar but for the exposure and confounding variables that we have recorded. Another limitation may be the association of the exposure location with the subject’s home. Although the majority of the time was spent by each adolescent in the house (17 h on average), another 5 h were spent at school and 2 h outdoors, including road transportation. Different exposure levels, especially for airborne particles, may have characterized the different sites. Nevertheless, even considering these limitations, the study indicates that living in an environment characterized by long-term exposure to metals – particularly Mn, can lead to impairment in the development of motor and olfactory functions that may be potentially considered as an early warning for the onset of late neurodegenerative effects in the older age. Preventive strategies toward sustainable soil should be promoted for urban areas close to metal emission from industries and further study should clarify the soil chemistry to allow better understanding of metal solubility, transport and bio-accessibility to plants and living organisms. Further research is scheduled in the Province of Brescia in areas close to metal emission from industries and further study should clarify the soil chemistry to allow better understanding of metal solubility, transport and bio-accessibility to plants and living organisms. Further research is scheduled in the Province of Brescia and manganese exposmes in Indianapolis, Indiana. J Expo Anal Environ Epidemiol 2010;20(1):45–50.

In summary, this is the first study showing association of manganese in surface soil and airborne particles with motor coordination, hand dexterity and odor identification and of manganese in blood and hair with tremor, in adolescents residing in areas with previous long term emission of metals – including manganese – from ferroalloy emission. The measurement of manganese in soil may represent an indicator of historical cumulative exposure.

Conflict of Interest statement

Zimmerman had paid expert testimony/consulting for plaintiff manganese exposure welder/welding rod lawsuits, and all the remainder of authors had declared no conflict of interest.

Acknowledgements

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References


