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Depression and anxiety in a manganese-exposed community

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ABSTRACT

Objective: To characterize the association between residential environmental manganese (Mn) exposure and depression and anxiety, given prior associations among occupationally-exposed workers.
Methods: We administered the Beck Depression Inventory (BDI) and the State-Trait Anxiety Inventory (STAI) to 697 study participants in their preferred languages. These participants represented a population-based sample of residents aged ≥40 from two predominantly Black African communities in Gauteng province, South Africa: 605 in Meyerton, adjacent to a large Mn smelter, and 92 in Ethembalethu, a comparable non-exposed community. We investigated the associations between community (Meyerton vs. Ethembalethu) and severity of depression and anxiety, using linear regression, adjusting for age and sex. To document community-level differences in Mn exposure, we measured airborne $PM_{2.5}$-Mn.
Results: Meyerton residents had BDI scores 5.63 points (95 % CI 3.07, 8.20) higher than Ethembalethu residents, with all questions contributing to this significant difference. STAI-state scores were marginally higher in Meyerton than Ethembalethu residents [2.12 (95 % CI -0.17, 4.41)], whereas STAI-trait scores were more similar between the communities [1.26 (95 % CI -0.82, 3.35)]. Mean $PM_{2.5}$-Mn concentration was 203 ng/m^3 at a long-term fixed site in Meyerton and 10 ng/m^3 in Ethembalethu.
Conclusion: Residence near Mn emission sources may be associated with greater depression symptomatology, and possibly current, but not lifetime, anxiety.

1. Introduction

Manganese (Mn) is an essential trace element but can be a neurotoxicant at higher levels (Aschner, 2000; Rodier, 1955; Wang et al., 1989). Routes of entry are oral, respiratory, and, possibly, trans-olfactory. Mn that bypasses the liver is actively transported across the blood-brain barrier and appears to accumulate in the basal ganglia (Nelson et al., 1993). Millions of people worldwide are exposed to airborne environmental Mn due to fossil fuel combustion, air erosion of Mn-laden soils proximate to mining operations, and industrial stack emissions from high temperature industrial processes, such as smelting and steelmaking (O'Neal and Zheng, 2015). Numerous studies demonstrate an association between occupational Mn exposure and motor dysfunction (Bouchard et al., 2007a, b; Bouchard et al., 2008; Roels et al., 1985), notably parkinsonism (Dlamini et al., 2020; Ma et al., 2018; Racette et al., 2017), and associated dysfunction of dopaminergic brain pathways (Criswell et al., 2018a, b; Criswell et al., 2020). While the dopaminergic system is critical for motor function, dopaminergic mesolimbic pathways are also important for mood homeostasis (Salamone, 1992; Salamone et al., 2005).

Studies in research participants occupationally-exposed to Mn, such as welders and smelter workers, consistently demonstrate effects on mood, i.e., depression and anxiety (Bast-Pettersen et al., 2004; Bowler et al., 2003,

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2007; Myers et al., 2003; Park, 2013). These studies generally suggest a modest dose-response association between occupational Mn exposure and both depression and anxiety. There is also modest evidence of an association between environmental Mn exposure and mood in children (Khan et al., 2011) and adults (Bowler et al., 2012; Donaldson, 1987; Sassine et al., 2002). Most notably, one study of environmentally-exposed residents of Marietta, OH, (exposed to airborne Mn from smelting operations) and a reference community, Mount Vernon, OH, found that Mn-exposed residents had higher generalized anxiety "scores" than non-exposed residents, as well as a relationship between anxiety and cumulative Mn exposure. There was no difference in symptoms of depression between the two communities (Bowler et al., 2012). In the current study, we sought to quantify the association between environmental Mn exposure and both anxiety and depression in a large, population-based study (Racette et al., 2021) of predominantly Black African residents from two communities with substantially different environmental Mn levels.

2. Methods

2.1. Participant recruitment

The Washington University School of Medicine Human Research Protection Office (St. Louis, Missouri, U.S.) and the University of the Witwatersrand Human Research Ethics Committee (Johannesburg, Gauteng, South Africa) approved this study. We developed this study protocol in collaboration with members of the community who brought their concerns, regarding air pollution from a smelter, to our attention, with the goal of improving air quality in their community. All participants were aware that the purpose of this study was to investigate the neurologic effects of environmental Mn exposure. Research participants were consented for participation in the privacy of their homes, and confidentiality of personal information was assured.

We recruited a population-based research sample from each of two communities in Gauteng province, South Africa, at the time of enrollment (2016–2020): one community with environmental Mn exposure (Meyerton) and one reference community (Ethembalethu). Research personnel who recruited participants were all residents of the Meyerton community. Participants in the Mn-exposed community, Meyerton, lived in one of three settlements: Old Sicelo, New Sicelo, or Noldick. The community of Meyerton is located within 5 km of one of the world's largest Mn smelters, which has been in operation since 1951. Participants from the reference community lived in Ethembalethu, a settlement located approximately 70 km northwest of Meyerton, with no nearby Mn smelting or mining operations. We chose Ethembalethu as the reference settlement due to its location in a non-industrial area, outside of Johannesburg, and similar demographics of residents, including race and socio-economic status. Most notably, the selected Meyerton-based and Ethembalethu settlements are government-subsidized housing communities, thus, residents must meet the same income criteria to be allowed to live in these settlements.

Study inclusion criteria were current residence in one of the two settlements and age ≥40 years. Trained field workers recruited research participants by visiting a preselected, population-based sample of homes in each settlement ("phase 1"). For two of the three Meyerton-based settlements (New Sicelo and Noldick), we preselected every second residence, using a municipality map. Research personnel invited eligible adults in each residence to participate in the study. If no one was home, or if there were no eligible adults in the residence, the residence to the left of the preselected home was visited. If no one was home or eligible in that residence, the next preselected home on the map was visited. Because there were fewer residences in Old Sicelo than in the other two settlements, eligible participants from every residence in that settlement were invited to participate. The reference community, Ethembalethu, was smaller than the Meyerton-based settlements, so we attempted to recruit every adult resident who met the study criteria, again using a similar door-to-door approach. We did not select participants from either community with regard to any health outcomes or occupational exposures, and generally, participants only had non-occupational exposure to Mn.

2.2. Assessment of Mn exposure

We used community (Meyerton, Ethembalethu) as an indicator of Mn exposure status. To quantify airborne Mn exposure, we measured ambient Mn concentrations in both communities over periods of several months. We collected fine particulate matter ($PM_{2.5}$ particles with aerodynamic diameter ≤2.5 μm) on Teflon® filters (Measurement Technology Corporation, Minneapolis, MN), using air samplers with $PM_{2.5}$ inlets (Model PQ100, Mesa Labs, Butler, NJ) operating continuously for two- to three-days for each sample. Long-term routine air sampling at a fixed site in the Meyerton settlement of Noldick began in October 2015 and was completed in May 2018. For the two-year period 2016–2017, 47 % of all hours were represented (n = 158 filters). We assessed spatial variability across the Meyerton-based settlements by collecting samples concurrently in Old Sicelo and Noldick (October 2018-February 2019, n = 37 filters), and New Sicelo and Noldick (September 2017-May 2018 and October 2018, n = 55 filters). We conducted air sampling in Ethembalethu in January-October 2020 (n = 68 filters) with no concurrent sampling in Meyerton. Filter membranes were digested using a MARS 6™microwave digestion system (CEM, Matthews, NC), using a validated protocol (Kulkarni et al., 2007). We filtered these digestates through 0.45 μm (pore size) nylon syringe filters (VWR, Radnor, PA) and diluted them with deionized water (≥18.2 MΩ/cm resistivity, MilliQ Water Purification System, EMD Millipore, Burlington, MA). Mn was quantified using an inductively coupled plasma-mass spectrometer (NexION® 2000, Perkin-Elmer, Norwalk, CT); the limit of detection was 0.056 ng/m^3 in $PM_{2.5}$ (Occupational Safety and Health Administration (OSHA), 2011). Instrument performance was validated using NIST 1648a Urban Particulate Matter (Sigma-Aldrich, St. Louis, MO), yielding Mn recovery of 96.9 ± 8.4 %.

2.3. Assessment of depression and anxiety

2.3.1. Instruments

To assess depression and anxiety, we administered the Beck Depression Inventory (BDI) II, a 21-item self-rated questionnaire that measures severity of depressive symptoms (Beck and Steer, 1987) and the State-Trait Anxiety Inventory (STAI), a 40-item self-rating scale for current anxiety symptoms (state) and lifetime anxiety symptoms (trait) (Spielberger et al., 1983). Each question item response is reported by the respondent on a 4-point Likert scale, ranging from 0–3 for the BDI and 1–4 for the STAI. The BDI questions are worded in a negative manner, with higher values indicating greater depression. Some of the anxiety (STAI) questions are worded positively (e.g., "I feel calm") and some negatively (e.g., "I feel tense"). For consistency and interpretability of results, we recoded the STAI question responses per standard practice, such that higher values always indicate greater anxiety. We then summed the BDI responses to obtain the BDI score and the STAI responses to obtain the STAI score: specifically questions 1–20 for the STAI-state score and questions 21–40 for the STAI-trait score. Thus, higher scores on both of these instruments indicated greater levels of depression/anxiety consistently. These standardized instruments were not available in the most common languages spoken in Gauteng province. Therefore, as detailed below, we translated and validated the BDI and STAI prior to using them in our study.

2.3.2. Translation of the BDI and STAI

We first modified and translated the English versions of the questionnaires, in accordance with previously described methods (Aaronson et al., 1992). We modified the questions without changing the original intent but to ensure easy comprehension by English-speaking South Africans (Nelson et al., 2020). The modified English language questionnaires were reviewed for accuracy and appropriateness, after which individuals proficient in four of the other most common languages in South Africa (Afrikaans, Setswana, Sesotho, and IsiZulu) translated them. We opted not to make additional translations of the Nguni language Sepedi, which is a combination of Setswana and Sesotho, or to IsiXhosa, which is linguistically similar to IsiZulu. The questionnaires were back translated by different individuals, also proficient in the respective languages.

In the second step (content validation), we tested the performance of the modified and/or translated questionnaires in 20 (four for each language), English-, Afrikaans-, Setswana-, Sesotho-, and IsiZulu-speaking male and female volunteers with varying educational levels. Where necessary, further modifications were made for all languages. Interviewers who were fluent in one or more of the relevant languages were trained by skilled qualitative researchers to administer the questionnaires, face-to-face. The interviews were conducted in accordance with previously outlined methodology (Nelson et al., 2020). Based on the results of these cognitive interviews, we made additional minor changes to the questionnaires. These linguistic alterations were required to ensure semantic and cultural equivalence by using relevant "language/words" as commonly understood in the described context/culture. For example, in the STAI questionnaire "I feel steady" was revised to "I feel emotionally stable," and "I am presently worrying over possible misfortunes" was revised to "I am presently worrying over possible setbacks."

2.3.3. Validation of the BDI and STAI

We recruited a convenience sample of 200 individuals aged ≥40 years, in order to validate translated versions of the standardized depression and anxiety instruments (hereafter referred to as the "validation sample"). The 200 individuals (40 per language) spoke one of five languages: Afrikaans, Setswana, Sesotho, IsiZulu, or South African English. These individuals lived in a residential area in the Midvaal region, which includes the Meyerton settlements, but were independent of the research sample. The majority (87.1 %) of participants in this validation sample completed some secondary or higher education (Supplemental Table 1). Most participants spoke Sesotho (37.6 %), English (21.5 %), or Afrikaans (19.9 %). A quarter had moderate to severe depression, and just over half had high anxiety according to both the state and trait portions of the STAI (Supplemental Table 2).

Using the BDI and STAI questionnaire responses, we calculated Cronbach's alpha coefficient on standardized values of the individual questions as a measure of internal consistency. This is a measure of relatedness of items in a group. We considered alpha coefficients ≥0.70 as acceptable for application in an epidemiological study (Tavakol and Dennick, 2011) and performed factor analysis (Nelson et al., 2020). We retained factors with an eigenvalue >1.0. In order to characterize each factor, we also determined factor loadings to identify which questions had a correlation >0.30 with each factor.

The overall internal consistency was high for the BDI (Cronbach's alpha = 0.93), as was that for each language (Cronbach's alpha = 0.88−0.89 in Setswana and IsiZulu, and 0.95−0.96 in Afrikaans, English, and Sesotho). Internal consistency also was high for the state (Cronbach's alpha = 0.86) and the trait (Cronbach's alpha = 0.86) parts of the STAI questionnaire. There was some variation by language (Cronbach's alpha for these were 0.93−0.95 state and trait for Afrikaans and English; 0.68 for STAI-state and 0.76 for STAI-trait in Setswana; 0.84 for STAI-state and 0.78 for STAI-trait in Sesotho; and 0.81 for STAI-state and 0.68 for STAI-trait in IsiZulu). Similarly, internal consistency for the BDI was essentially the same across groups defined by educational attainment (Cronbach's alpha = 0.93−0.95), but was more variable for each of the two parts of the STAI (Cronbach's alpha = 0.91) for individuals with grade 12 and higher levels of education and (Cronbach's alpha = 0.83−0.84) for lower levels of education. There was no clear difference between those with a secondary vs. primary or lower educational attainment.

The factor analysis for BDI retained only one factor (eigenvalue = 8.3), and all questions loaded on this factor (all factor loadings >0.30). We interpreted this one-factor structure as consistent with validation of the BDI (Huang and Chen, 2015). Factor analysis for the STAI revealed three factors. Two factors (eigenvalues 12.4 and 5.3) together accounted for 93.0 % of the total variance (65.1 % and 27.9 %, respectively). All questions loaded with a correlation >0.30 on only one of these two factors. Specifically, all of the STAI questions worded in a positive manner loaded onto the first factor, whereas all STAI questions worded in a negative manner loaded onto the second factor. The separation of these primary two factors according to positive vs. negative wording of questions occurred regardless of language version. The third, more minor factor (eigenvalue = 1.33, 7.0 % of the variance) loaded on 8/10 of the negatively-worded STAI-state questions, but no other questions (no positively-worded STAI-state questions and no STAI-trait questions). This three-factor result aligned very well with a four-factor structure of the STAI (state-positive questions, state-negative questions, trait-positive questions, and trait-negative questions) that has been shown to be a better fit than the simple two-factor (state-trait) structure (Vigneau and Cormier, 2008).

2.3.4. Assessment of BDI and STAI in research participants

We administered the validated BDI and STAI instruments to research participants. Participants could complete the instruments on their own in the language of their choice or receive assistance from trained study staff. Staff administered the BDI and STAI at a second, clinical ("phase 2″) visit that occurred after recruitment. The phase 2 visit took place at a local community center and included an examination by a neurologist. We attempted to optimize participant privacy by administering the questionnaires in separate rooms in a central location in each settlement (Meyerton and Ethembalethu).

2.4. Statistical analysis

We used Stata (StataCorp, 2015) to perform linear regression analysis to assess the association between Mn exposure (community) and depression and anxiety. Our primary outcomes were the raw BDI or STAI score, with the state and trait portions of the STAI questionnaire considered separately. To compare the magnitude of the linear regression β-coefficient estimates for these three outcomes, while preserving associations as observed without rescaling, we also transformed the BDI, STAI-state, and STAI-trait scores to z-scores, representing standard deviations from the mean for the respective questionnaires. This permitted comparison of the magnitude of the linear regression β-coefficient estimates for these three outcomes. Secondarily, we considered BDI and STAI subscores, i.e., responses to the individual questions as outcomes. We retained each of the BDI and STAI outcomes as continuous dependent variables. Mn exposure, as assessed by whether the residence was in the exposed (Meyerton) or non-exposed (Ethembalethu) community, was the independent variable of interest. Given the known association between age (Beekman et al., 2000; Sami and Nilforooshan, 2015) and sex (Leach et al., 2008; Seney and Sibille, 2014) and both depression and anxiety, we adjusted *a priori* for age and sex in all models. We retained age as a continuous variable and adjusted for age using natural cubic splines with five knots, following Harrell's placement method (Harrell, 2015), as we applied previously (Racette et al., 2021). We further examined the effect of adjusting for education, body mass index (BMI), smoking, and consumption of alcohol, as potential confounding variables. Specifically, we adjusted for education as a categorical variable (none/non-formal schooling, primary, secondary, grade 12/higher); BMI, measured at the time of assessment, as a categorical variable (<18.5, 18.5–24.9, 25.0–29.9, ≥30) and verified results as similar with a dichotomous variable (<25, ≥25); and smoking and alcohol use as trichotomous variables (never, former, current) and verified results as similar with a dichotomous variable (never, ever). We did not adjust for these variables *a priori*. Although these variables could act as true confounders for the association between Mn exposure (community) and depression/anxiety, it is also plausible that they could mediate the association or, alternatively, that depression/anxiety could affect educational attainment, BMI, smoking, and alcohol consumption (i.e., be mediators rather than outcomes). In other words, it was unclear if it was appropriate to adjust for any of these variables. For all regression analyses, we considered a two-sided p-value of 0.05 as statistically significant, as evidenced by the exclusion of zero from the 95 % confidence interval (CI) for the β-coefficient, i.e., adjusted mean difference between Meyerton and Ethembalethu. Finally, because we assessed depression and anxiety using questionnaires, we explored whether the associations between community and depression/anxiety differed by home language. We categorized home language into six categories, namely, the five most commonly observed home languages in the research sample (Setswana, Sesotho, Sepedi, IsiXhosa, and IsiZulu) and one additional group with all other languages combined, including Afrikaans and English. Similarly, we considered all potential confounders as potential effect modifiers. We compared the β-coefficients, and then formally tested for interaction in regression models with a likelihood ratio test comparing two models (with and without the interaction terms) or by including a single product term in a single model, when possible.

3. Results

3.1. Research participant characteristics

Out of the 666 homes that we visited in Meyerton, 462 (69.4 %) had at least one age-eligible adult who agreed to participate, and out of the 108 homes we visited in Ethembalethu, 79 (73.1 %) had at least one age-eligible adult who agreed to participate. Initially, we recruited 832 eligible participants (732 in Meyerton, 100 in Ethembalethu) (Fig. 1). The median time between the first (enrollment ~ phase 1) and second (phase 2) visits in Meyerton and Ethembalethu was 49 and 3 days, respectively. Accordingly, although most participants attended the phase 2 visit where the BDI and STAI were administered, slightly more did so in Ethembalethu (85.9 % in Meyerton and 96.0 % in Ethembalethu). We excluded an additional 24 participants from Meyerton and four from Ethembalethu who had missing subscores for the BDI and/or STAI or who did not complete one or both tests, despite completing other phase 2 study visit tests. Thus, we included 605 participants from Meyerton and 92 participants from Ethembalethu (Fig. 1). These participants were similar to the participants from the respective community who were enrolled but did not attend the phase 2 visit or had no BDI/STAI data with respect to demographic variables, including home language (all $p > 0.05$, not shown).

Most participants in both communities were Black (98.8 % in Meyerton and 97.8 % in Ethembalethu) (Table 1). A majority of participants in both communities were women (53.4 % in Meyerton and 67.4 % in Ethembalethu). Median age of participants was 49 in Meyerton and 55 in Ethembalethu. Thirteen (2.1 %) Meyerton residents had ever worked in jobs with potential Mn exposure; no Ethembalethu residents had occupational Mn exposure.

3.2. Air Mn concentration in the study communities

The two-year (2016–2017) mean $PM_{2.5}$-Mn concentration from the long-term particulate matter air sampling in the Noldick settlement of Meyerton was 203 ng/m^3. This mean was approximately twice that for both Old Sicelo and New Sicelo; the $PM_{2.5}$-Mn ratios of means were 0.45 at Old Sicelo and 0.65 at New Sicelo, compared to Noldick. The $PM_{2.5}$-Mn mean concentration in Ethembalethu (year 2020) was 10 ng/m^3, i.e., ~20 times lower than the mean concentration in Noldick. Air $PM_{2.5}$-Mn concentration at a long-term fixed site in Meyerton differed over time, with both decreasing levels over study time and variation by season, with highest levels in the winter.

3.3. Mn exposure and depression

A higher proportion of Meyerton (38.1 %) than Ethembalethu (19.6 %) residents had moderate to severe depression (Table 2). Meyerton residents had BDI scores that were 5.63 (95 % CI 3.07, 8.20) points higher (worse) than those for Ethembalethu residents, which represented a 0.49 (95 % CI 0.26, 0.71) difference in z-scores between the two communities (Table 3). Further adjustment for education, smoking, alcohol, or BMI attenuated these differences only modestly (Table 3),

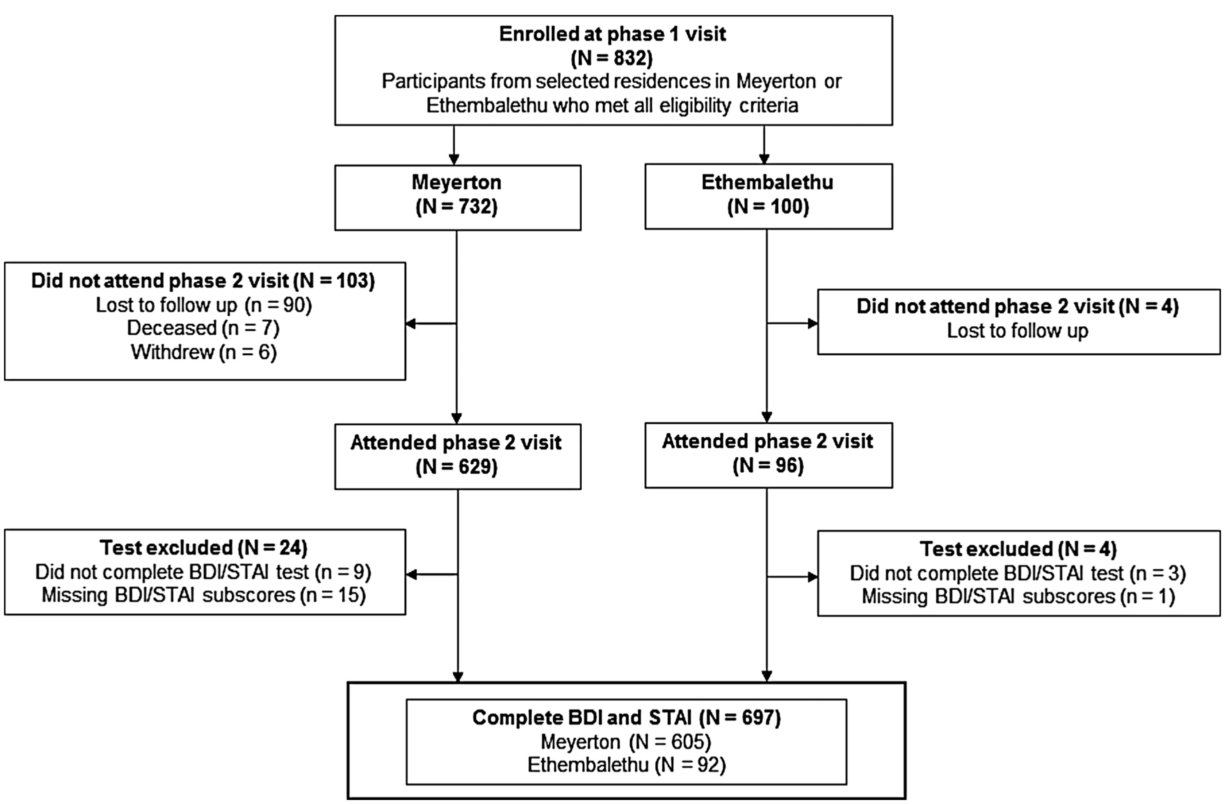

Fig. 1. Study participants, Gauteng province, South Africa, 2016-2020. Participating residents in our study sample (N = 697) from Meyerton (n = 605) and Ethembalethu (n = 92). Eligible participants were aged ≥40.
Abbreviations: BDI=Beck Depression Inventory; STAI=State-Trait Anxiety Inventory.

Table 1
Participant characteristics by community, Gauteng province, South Africa, 2016-2020.

	All participants n = 697 n (%)	Mn-exposed community (Meyerton) n = 605 n (%)	Reference community (Ethembalethu) n = 92 n (%)
Sex			
Male	312 (44.8)	282 (46.6)	30 (32.6)
Female	385 (55.2)	323 (53.4)	62 (67.4)
Race[a]			
Black	687 (98.7)	598 (98.8)	89 (97.8)
Other	9 (1.3)	7 (1.2)	2 (2.2)
Language[b]			
Sesotho	355 (51.2)	341 (56.6)	14 (15.2)
IsiXhosa	109 (15.7)	101 (16.8)	8 (8.7)
IsiZulu	103 (14.8)	89 (14.8)	14 (15.2)
Setswana	42 (6.1)	13 (2.2)	29 (31.5)
Sepedi	22 (3.2)	17 (2.8)	5 (5.4)
Afrikaans/ English/other	63 (9.1)	41 (6.8)	22 (23.9)
Education[c]			
None/non-formal schooling	102 (14.6)	94 (15.5)	8 (8.7)
Primary	263 (37.7)	223 (36.9)	40 (43.5)
Secondary	228 (32.7)	200 (33.1)	28 (30.4)
Grade 12 or higher	104 (14.9)	88 (14.6)	16 (17.4)
Smoking cigarettes			
Never	478 (68.6)	399 (66.0)	79 (85.9)
Former	57 (8.2)	56 (9.3)	1 (1.1)
Current	162 (23.2)	150 (24.8)	12 (13.0)
Alcohol use			
Never	355 (50.9)	292 (48.3)	63 (68.5)
Former	110 (15.8)	99 (16.4)	11 (12.0)
Current	232 (33.3)	214 (35.4)	18 (19.6)
BMI[d]			
<18.5	45 (6.5)	44 (7.3)	1 (1.1)
18.5–24.9	234 (33.7)	215 (35.7)	19 (20.7)
25.0–29.9	150 (21.6)	122 (20.2)	28 (30.4)
≥30	266 (38.3)	222 (36.8)	44 (47.8)
Ever Mn occupational exposure	13 (1.9)	13 (2.1)	0 (0.0)
Current Mn occupational exposure	2 (0.3)	2 (0.3)	0 (0.0)
	Mean (SD)	Mean (SD)	Mean (SD)
Age, years	51.8 (9.2)	51.2 (9.2)	55.6 (8.9)
Minimum	40	40	40
Median	50	49	55
Maximum	89	89	84

Abbreviations: BMI=body mass index; Mn=Manganese; SD=standard deviation.
[a] Percent excludes 1 participant from Ethembalethu with missing data. Other is White or of mixed race.

[b] Percent excludes 3 participants from Meyerton with missing data. Other languages included Xitsonga, SiSwati, Tshivenda, and IsiNdebele.

[c] Primary is grades 1–7, secondary is grades 8–11.

[d] Excludes 2 participants from Meyerton with missing data.

and we confirmed that season also did not confound the association between community and depression. The association between community and depression was found in both men (7.66, 95 % CI 3.19, 12.13) and women (4.75, 95 % CI 1.61, 7.88), and evident in five of the six home language groups (all except IsiXhosa, data not shown). We did not observe the association between community and BDI score in participants in the oldest age category (≥60 years) and those in the lowest category of education. On average, all BDI subscores were higher in the Meyerton than Ethembalethu residents, the majority of which represented significant differences (Table 4).

3.4. Mn exposure and anxiety

Slightly more than one third of participants from both communities had high state anxiety scores (37.5 % in Meyerton and 35.9 % in Ethembalethu) (Table 2). After adjusting for age and sex, STAI-state scores were marginally higher (worse) in Meyerton than Ethembalethu

Table 2
BDI and STAI by community, Gauteng province, South Africa, 2016-2020.

	All participants n = 697 n (%)	Mn-exposed community (Meyerton) n = 605 n (%)	Reference community (Ethembalethu) n = 92 n (%)
BDI			
Depression severity[a]			
Minimal	304 (43.6)	249 (41.2)	55 (59.8)
Mild	144 (20.7)	125 (20.7)	19 (20.7)
Moderate	124 (17.8)	114 (18.8)	10 (10.9)
Severe	125 (17.9)	117 (19.3)	8 (8.7)
Score			
Mean (SD)	17.3 (11.6)	17.9 (11.8)	13.0 (9.4)
Minimum	0	0	0
Median	15	16	11
Maximum	56	56	49
STAI-state[b]			
High state anxiety[c]	260 (37.3)	227 (37.5)	33 (35.9)
Score			
Mean (SD)	37.2 (10.2)	37.4 (10.3)	35.5 (9.5)
Minimum	20	20	20
Median	36	36	34
Maximum	73	73	63
STAI-trait[d]			
High trait anxiety[c]	421 (60.4)	372 (61.5)	49 (53.3)
Score			
Mean (SD)	42.6 (9.2)	42.8 (9.2)	41.9 (9.5)
Minimum	21	21	24
Median	44	44	41.5
Maximum	69	69	68

Abbreviations: BDI=Beck Depression Inventory; Mn=manganese; SD=standard deviation; STAI=State-Trait Anxiety Inventory.
[a] BDI score severity rating scale: minimal = 0–13, mild = 14–19, moderate = 20–28, severe = 29–63.

[b] Evaluates the current state of anxiety, how respondents “feel right now, that is, at this moment”; STAI questionnaire items 1–20.

[c] STAI score >40.

[d] Evaluates how respondents “generally feel”; STAI questionnaire items 21–40.

Table 3
BDI and STAI scores in the study sample in relation to community, Gauteng province, South Africa, 2016-2020.

	Difference in mean scores[a] between Mn-exposed (Meyerton) and reference community (Ethembalethu) (95 %CI)[b]					
	Unadjusted	Age/sex-adjusted	Age/sex/education adjusted	Age/sex/BMI-adjusted	Age/sex/smoking/alcohol-adjusted	Age/sex/education/BMI/smoking/alcohol-adjusted
Total score						
BDI	4.91 (2.39, 7.43)	5.63 (3.07, 8.20)	5.21 (2.63, 7.79)	5.39 (2.80, 7.97)	5.33 (2.74, 7.92)	4.82 (2.20, 7.43)
STAI-state[c]	1.87 (-0.37, 4.10)	2.12 (-0.17, 4.41)	1.59 (-0.71, 3.88)	1.63 (-0.66, 3.92)	1.83 (-0.48, 4.14)	0.99 (-1.33, 3.30)
STAI-trait[d]	0.85 (-1.19, 2.88)	1.26 (-0.82, 3.35)	0.78 (-1.30, 2.86)	0.87 (-1.21, 2.96)	1.00 (-1.11, 3.10)	0.29 (-1.81, 2.40)
Z-score[e]						
BDI	0.42 (0.21, 0.64)	0.49 (0.26, 0.71)	0.45 (0.23, 0.67)	0.47 (0.24, 0.69)	0.46 (0.24, 0.68)	0.42 (0.19, 0.64)
STAI-state[c]	0.18 (-0.04, 0.40)	0.21 (-0.02, 0.43)	0.16 (-0.07, 0.38)	0.16 (-0.06, 0.39)	0.18 (-0.05, 0.41)	0.10 (-0.13, 0.32)
STAI-trait[d]	0.09 (-0.13, 0.31)	0.14 (-0.09, 0.36)	0.08 (-0.14, 0.31)	0.09 (-0.13, 0.32)	0.11 (-0.12, 0.34)	0.03 (-0.20, 0.26)

Abbreviations: BDI=Beck Depression Inventory; BMI=body mass index; CI=confidence interval; STAI=State-Trait Anxiety Inventory.

[a] Higher scores indicate greater levels of depression/anxiety. The anxiety scores are standardized such that higher values always indicate greater anxiety.

[b] Mean differences (β-coefficient estimates) from linear regression comparing the BDI and STAI scores (outcome variables) in Meyerton (Mn-exposed community) relative to Ethembalethu (reference community). Age adjustment was made using natural cubic splines with five knots (5th, 27.5th, 50th, 72.5th, and 95th percentiles) as per Harrell's placement method (Harrell, 2015); education adjustment was as a categorical variable (none/non-formal schooling, primary, secondary, grade 12/higher); smoking and alcohol adjustment was as a trichotomous variable (never, former, current) and verified as similar with a dichotomous variable (never, ever); and BMI adjustment was as a categorical variable (<18.5, 18.5–24.9, 25.0–29.9, ≥30) and verified as similar with a dichotomous variable (<25, ≥25). All results are based on 697 participants (605 in Meyerton and 92 in Ethembalethu) with the following exception: excludes 2 participants from Meyerton with missing BMI data in models with adjustment for the respective variable.

[c] Evaluates the current state of anxiety, how respondents "feel right now, that is, at this moment"; STAI questionnaire items 1–20.

[d] Evaluates how respondents "generally feel"; STAI questionnaire items 21–40.

[e] We transformed the BDI and STAI scores to z-scores representing standard deviations from the mean within the present sample for the respective questionnaires so that the magnitude of the β-coefficient estimates could be compared across questionnaires.

residents: 2.12 (95 % CI -0.17, 4.41) higher absolute scores, and 0.21 (95 %CI -0.02, 0.43) higher z-scores (Table 3). This possible association was somewhat stronger in men than in women, and in younger than in older age groups. However, education was the only variable for which we observed significant interaction between community and STAI-state scores. Specifically, there was no association between community and STAI-state scores in either of the two lower education categories (-0.35, 95 % CI -3.46, 2.76, when combined), which contrasted starkly with the other two (higher) education categories when combined (4.61, 95 % CI 1.22, 8.01; interaction p-value = 0.01).

The overall association between community and STAI-state differed by home language, but the presence or strength of associations did not appear to vary as a function of Cronbach's alpha for the translated STAI questionnaires. Instead, we found that STAI-state scores were (non-significantly) higher in Meyerton than Ethembalethu residents for IsiXhosa and Sepedi language speakers, if we considered the positively-worded questions. In contrast, for Sesotho and Setswana language speakers, STAI-state scores were (non-significantly) higher in Meyerton than Ethembalethu residents, if we considered the negatively-worded questions. There was no association between community and STAI-state for IsiZulu or "other" language speakers. Associations for trait anxiety largely paralleled those for state anxiety, but were weaker (Table 3). In general, STAI-state subscores tended to be higher in Meyerton than Ethembalethu residents (Table 5). The associations between community and these questions, as well as the overall STAI-state and STAI-trait scores, were attenuated with adjustment for education, smoking, alcohol, and/or BMI (Tables 3 and 5), but not with adjustment for season of assessment.

3.5. *Cognitive and motor health effects as potential contributors to Mn-mood associations*

Given the association between community and depression, and potentially STAI-state, we conducted a *post hoc* mediation analysis in which we explored whether other potential health effects of Mn, namely motor or cognitive effects, might contribute to all or part of the observed associations for these two mood outcomes. Specifically we considered each individual's perception of severity of motor or cognitive issues as assessed by self-report using selected questions on the 39-item Parkinson Disease Questionnaire (PDQ-39). The question "Felt your memory was bad?" mediated 15.5 % (95 % CI 10.7, 27.7 %) of the association between Mn exposure and depression. Results were similar for the STAI-state, although the CIs were very wide. Accordingly, the point estimates for the Mn-BDI and Mn- STAI-state associations were attenuated somewhat with adjustment for the response to the PDQ-39 memory question (4.70, 95 % CI 2.08, 7.32 and 1.70, 95 % CI -0.65, 4.06, respectively). In contrast, only approximately 6–7 % of the Mn-mood associations were mediated by having difficulty with selected activities of daily living (assessed as a summary score based on difficulty carrying shopping bags, walking around the house or neighborhood, falling in public, dressing, holding a drink, or with speech).

4. Discussion

The findings from this study provide evidence of an association between environmental air Mn exposure and mood dysfunction, notably more severe symptoms of depression. For example, the magnitude of the observed differences in depression between study participants from the

Table 4
BDI subscores, by community, Gauteng province, South Africa, 2016-2020.

	Question description	Mean (SD)[a]		Mean difference (95 %CI)[b]	
		Mn-exposed community (Meyerton)	Reference community (Ethembalethu)	Age/sex-adjusted	Age/sex/education/BMI/smoking/alcohol-adjusted
BDI 1	Sadness	0.71 (1.04)	0.39 (0.80)	0.29 (0.06, 0.52)	0.29 (0.06, 0.52)
BDI 2	Pessimism	0.70 (1.03)	0.35 (0.75)	0.36 (0.13, 0.58)	0.33 (0.10, 0.56)
BDI 3	Past failure	0.89 (1.08)	0.49 (0.88)	0.43 (0.19, 0.67)	0.39 (0.15, 0.64)
BDI 4	Loss of pleasure	1.05 (1.00)	0.89 (0.92)	0.20 (-0.02, 0.43)	0.12 (-0.11, 0.34)
BDI 5	Guilty feelings	0.72 (0.93)	0.64 (0.72)	0.09 (-0.11, 0.29)	0.04 (-0.17, 0.24)
BDI 6	Punishment feelings	0.91 (1.20)	0.73 (1.01)	0.11 (-0.15, 0.38)	0.05 (-0.22, 0.32)
BDI 7	Self-dislike	0.44 (0.79)	0.22 (0.55)	0.19 (0.02, 0.36)	0.18 (0.001, 0.36)
BDI 8	Self-criticalness	0.78 (1.08)	0.48 (0.90)	0.23 (-0.01, 0.47)	0.21 (-0.03, 0.46)
BDI 9	Suicidal thoughts or wishes	0.24 (0.61)	0.08 (0.27)	0.15 (0.02, 0.28)	0.12 (-0.02, 0.25)
BDI 10	Crying	1.07 (1.21)	0.59 (1.05)	0.49 (0.22, 0.76)	0.48 (0.21, 0.76)
BDI 11	Agitation	0.83 (1.10)	0.62 (0.95)	0.23 (-0.01, 0.47)	0.19 (-0.06, 0.44)
BDI 12	Loss of interest	0.82 (1.08)	0.46 (0.88)	0.44 (0.20, 0.67)	0.43 (0.19, 0.67)
BDI 13	Indecisiveness	0.65 (0.94)	0.40 (0.79)	0.23 (0.03, 0.44)	0.18 (-0.03, 0.39)
BDI 14	Worthlessness	0.72 (0.95)	0.35 (0.70)	0.37 (0.16, 0.58)	0.30 (0.09, 0.51)
BDI 15	Loss of energy	1.14 (0.93)	0.78 (0.81)	0.48 (0.28, 0.67)	0.41 (0.21, 0.62)
BDI 16	Changes in sleeping pattern	1.20 (1.10)	1.04 (0.95)	0.22 (-0.02, 0.46)	0.26 (0.01, 0.50)
BDI 17	Irritability	0.73 (0.95)	0.48 (0.83)	0.28 (0.07, 0.49)	0.24 (0.02, 0.45)
BDI 18	Changes in appetite	1.00 (0.89)	0.91 (0.79)	0.14 (-0.06, 0.34)	0.10 (-0.11, 0.30)
BDI 19	Concentration difficulty	0.76 (0.95)	0.58 (0.73)	0.22 (0.02, 0.43)	0.21 (-0.003, 0.42)
BDI 20	Tiredness or fatigued	1.05 (1.03)	0.99 (0.91)	0.18 (-0.04, 0.40)	0.11 (-0.12, 0.33)
BDI 21	Loss of interest in sex	1.51 (1.20)	1.55 (1.27)	0.30 (0.06, 0.53)	0.19 (-0.05, 0.42)

Abbreviations: BDI=Beck Depression Inventory; BMI=body mass index; CI=confidence interval; SD=standard deviation.
[a] Higher scores indicate greater levels of depression. Responses are rated on a 4-point Likert scale and range from 0 to 3.

[b] Mean differences (β-coefficient estimates) from linear regression comparing the BDI subscores (outcome variables) in Meyerton (Mn-exposed community) relative to Ethembalethu (reference community). Age adjustment was made using natural cubic splines with five knots (5th, 27.5th, 50th, 72.5th, and 95th percentiles) as per Harrell's placement method (Harrell, 2015); smoking and alcohol adjustment was as a trichotomous variable (never, former, current) and verified as similar with a dichotomous variable (never, ever); BMI adjustment was as a categorical variable (<18.5, 18.5–24.9, 25.0–29.9, ≥30) and verified as similar with a dichotomous variable (<25, ≥25); and education as a categorical variable (none/non-formal schooling, primary, secondary, grade 12/higher). All results are based on 697 participants (605 in Meyerton and 92 in Ethembalethu) with the following exception: excludes 2 participants from Meyerton with missing BMI data in models with adjustment for the respective variable.

Mn-exposed and non-exposed communities (~5.5 points) was large enough to shift most BDI scores from the mild depression range to the moderate depression range. This difference could have a potentially substantial psychiatric public health impact, including exacerbation of co-morbid substance abuse. We also observed more modest potential differences in anxiety, specifically state anxiety, between the two study communities. These differences in mood are not likely due to age, sex, race or socio-economic status, given that our study was composed entirely of African residents from low-income, subsidized housing communities.

We confirmed marked Mn exposure contrasts between the two communities, although other air toxic metals, such as lead, were also higher in Meyerton than Ethembalethu, as would be expected in a community with an Mn smelter. Our study was hypothesis-based and focused on basal ganglia-mediated neuropsychological functions, which are mostly specific to Mn and not consistently associated with the other air toxic metals. We focused on $PM_{2.5}$-Mn because these particles are likely most relevant to adverse health outcomes. However, the historical literature is dominated by PM_{10} and even total suspended particulate (TSP) data. Mean $PM_{2.5}$-Mn concentrations among the three settlements in our exposed community were ~100−200 ng/m^3. For comparison, Hanoi $PM_{2.5}$-Mn for June 2015-December 2017 was 80 ng/m^3 with lower values observed at the other SPARTAN global PM network locations (McNeill et al., 2020). The Marietta, Ohio, USA study found mean $PM_{2.5}$-Mn concentrations of 45 ng Mn/m^3 (Bowler et al., 2012, 2016). Similarly, 16-month (2006–2007) mean $PM_{2.5}$-Mn was 77 ng/m^3 in Santander, Spain, located 7 km from a ferroalloy plant (Moreno et al., 2011). These examples demonstrate that a mega-city (Hanoi) and smaller cities influenced by a nearby Mn industrial emissions source can have long term $PM_{2.5}$-Mn levels approaching those observed in our study area. Finally, our low Mn-exposed community, Ethembalethu, had ambient $PM_{2.5}$-Mn levels that were below another non-industrial region in Durban, South Africa (mean $PM_{2.5}$-Mn = 17 ng/m^3) (Batterman et al., 2011).

Investigators in the Marietta study found significant differences in study participants reporting feeling "anxious" and "irritable," as well as in generalized anxiety, as measured by the sum of T-scores for obsessive-compulsive, anxiety, and phobic anxiety scales from the Symptom Checklist-90-Revised (SCL-90-R) (Bowler et al., 2012). However, in contrast to our study, they found no differences in a depression variable

Table 5
STAI subscores, by community, Gauteng province, South Africa, 2016-2020.

	Question description	Mean (SD)[a]		Difference in mean subscores, Mn-exposed vs. reference community (95 % CI)[b]	
		Mn-exposed community (Meyerton)	Reference community (Ethembalethu)	Age/sex-adjusted	Age/sex/education/BMI/smoking/alcohol-adjusted
STAI-state[c]					
STAI 1	I feel calm	1.91 (0.93)	1.65 (0.82)	0.28 (0.07, 0.48)	0.22 (0.01, 0.43)
STAI 2	I feel secure	1.78 (0.86)	1.66 (0.80)	0.10 (-0.10, 0.29)	−0.002 (-0.20, 0.19)
STAI 3	I am tense	2.07 (1.11)	1.88 (1.10)	0.24 (-0.01, 0.49)	0.21 (-0.05, 0.46)
STAI 4	I feel strained	2.07 (1.14)	1.97 (1.11)	0.18 (-0.07, 0.44)	0.12 (-0.14, 0.38)
STAI 5	I feel at ease	2.08 (0.99)	1.82 (0.96)	0.28 (0.06, 0.51)	0.22 (-0.01, 0.44)
STAI 6	I feel upset	1.54 (0.95)	1.58 (0.94)	−0.04 (-0.25, 0.17)	−0.06 (-0.28, 0.15)
STAI 7	I am presently worrying over possible setbacks	2.05 (1.15)	2.08 (1.16)	−0.05 (-0.31, 0.21)	−0.03 (-0.30, 0.23)
STAI 8	I feel satisfied	1.87 (0.97)	1.82 (0.96)	0.06 (-0.16, 0.28)	−0.02 (-0.24, 0.21)
STAI 9	I feel frightened	1.62 (0.98)	1.58 (0.94)	0.03 (-0.19, 0.25)	−0.03 (-0.26, 0.19)
STAI 10	I feel comfortable	1.78 (0.87)	1.77 (0.93)	0.02 (-0.18, 0.22)	−0.06 (-0.26, 0.14)
STAI 11	I feel self-confident	1.73 (0.94)	1.53 (0.73)	0.24 (0.04, 0.44)	0.12 (-0.08, 0.33)
STAI 12	I feel nervous	1.72 (0.97)	1.73 (1.00)	−0.02 (-0.24, 0.20)	−0.06 (-0.28, 0.17)
STAI 13	I am jittery	1.70 (0.96)	1.73 (0.90)	−0.04 (-0.25, 0.18)	−0.06 (-0.28, 0.16)
STAI 14	I feel indecisive	2.34 (1.21)	2.23 (1.06)	0.15 (-0.12, 0.42)	0.14 (-0.14, 0.41)
STAI 15	I am relaxed	1.80 (0.89)	1.92 (1.02)	−0.08 (-0.28, 0.13)	−0.14 (-0.35, 0.07)
STAI 16	I feel content	1.91 (0.96)	1.83 (0.96)	0.08 (-0.13, 0.30)	0.02 (-0.20, 0.25)
STAI 17	I am worried	1.79 (1.09)	1.64 (0.93)	0.16 (-0.08, 0.40)	0.10 (-0.15, 0.35)
STAI 18	I feel confused	1.56 (0.93)	1.36 (0.67)	0.19 (-0.01, 0.39)	0.12 (-0.09, 0.32)
STAI 19	I feel emotionally stable	2.19 (1.09)	2.07 (1.07)	0.14 (-0.10, 0.39)	0.08 (-0.17, 0.33)
STAI 20	I feel pleasant	1.91 (0.93)	1.72 (0.82)	0.20 (-0.005, 0.41)	0.10 (-0.11, 0.31)
STAI-trait[d]					
STAI 21	I feel pleasant	2.05 (0.87)	1.89 (0.72)	0.18 (-0.01, 0.37)	0.11 (-0.08, 0.31)
STAI 22	I feel nervous and restless	2.05 (0.96)	2.04 (0.99)	0.03 (-0.19, 0.24)	−0.01 (-0.23, 0.22)
STAI 23	I feel satisfied with myself	1.89 (0.98)	1.86 (0.90)	0.05 (-0.17, 0.27)	0.02 (-0.20, 0.25)
STAI 24	I wish I could be as happy as others seem to be	2.93 (1.04)	2.91 (1.03)	0.01 (-0.23, 0.24)	−0.08 (-0.31, 0.16)
STAI 25	I feel like a failure	1.98 (1.06)	1.70 (0.95)	0.34 (0.11, 0.58)	0.29 (0.05, 0.53)
STAI 26	I feel rested	2.20 (0.91)	2.01 (0.87)	0.22 (0.01, 0.42)	0.19 (-0.02, 0.40)
STAI 27	I am "calm, cool, and collected"	1.97 (0.89)	1.89 (0.79)	0.10 (-0.10, 0.30)	0.06 (-0.14, 0.26)
STAI 28	I feel that difficulties are piling up so that I cannot overcome them	2.25 (1.02)	2.02 (0.97)	0.27 (0.04, 0.50)	0.20 (-0.03, 0.44)
STAI 29	I worry too much over something that really doesn't matter	2.04 (0.96)	2.10 (1.05)	−0.01 (-0.23, 0.21)	−0.03 (-0.26, 0.19)
STAI 30	I am happy	1.86 (0.86)	1.89 (0.86)	−0.02 (-0.21, 0.17)	−0.05 (-0.25, 0.15)
STAI 31	I have disturbing thoughts	2.16 (1.05)	2.08 (0.99)	0.14 (-0.09, 0.38)	0.09 (-0.14, 0.33)
STAI 32	I lack self-confidence	2.34 (1.18)	2.00 (1.09)	0.38 (0.11, 0.64)	0.34 (0.07, 0.60)
STAI 33	I feel secure	1.93 (0.93)	2.05 (0.96)	−0.14 (-0.35, 0.07)	−0.24 (-0.45, -0.02)
STAI 34	I make decisions easily	2.08 (0.99)	2.16 (0.93)	−0.11 (-0.33, 0.11)	−0.14 (-0.36, 0.09)
STAI 35	I feel inadequate	2.22 (0.92)	2.26 (0.95)	−0.005 (-0.21, 0.20)	−0.02 (-0.24, 0.19)
STAI 36	I am content	2.04 (0.90)	2.01 (0.82)	0.02 (-0.18, 0.22)	−0.03 (-0.24, 0.17)
STAI 37	Some unimportant thought runs through my mind and bothers me	2.16 (0.95)	2.23 (0.98)	−0.06 (-0.28, 0.15)	−0.14 (-0.36, 0.08)

(*continued on next page*)

Table 5 (*continued*)

	Question description	Mean (SD)[a]		Difference in mean subscores, Mn-exposed vs. reference community (95 % CI)[b]	
		Mn-exposed community (Meyerton)	Reference community (Ethembalethu)	Age/sex-adjusted	Age/sex/education/BMI/ smoking/alcohol-adjusted
STAI 38	I take disappointments so keenly that I can't put them out of my mind	2.36 (1.01)	2.27 (0.98)	0.14 (-0.09, 0.37)	0.05 (-0.18, 0.28)
STAI 39	I am a stable person	1.97 (0.91)	2.10 (0.90)	−0.08 (-0.29, 0.12)	−0.14 (-0.35, 0.06)
STAI 40	I get in a state of tension or turmoil as I think over my recent concerns & interests	2.26 (0.93)	2.43 (0.98)	−0.18 (-0.39, 0.04)	−0.18 (-0.40, 0.03)

Abbreviations: BMI=body mass index; CI=confidence interval; SD=standard deviation; STAI=State-Trait Anxiety Inventory.

[a] Higher scores indicate greater levels of anxiety. The anxiety scores are recoded such that higher values always indicate greater anxiety. Responses are rated on a 4-point Likert scale and range from 1 to 4.

[b] Mean differences (β-coefficient estimates) from linear regression comparing the STAI subscores (outcome variables) in Meyerton (Mn-exposed community) relative to Ethembalethu (reference community). Age adjustment was made using natural cubic splines with five knots (5th, 27.5th, 50th, 72.5th, and 95th percentiles) as per Harrell's placement method (Harrell, 2015); smoking and alcohol adjustment was as a trichotomous variable (never, former, current) and verified as similar with a dichotomous variable (never, ever); BMI adjustment was as a categorical variable (<18.5, 18.5–24.9, 25.0–29.9, ≥30) and verified as similar with a dichotomous variable (<25, ≥25); and education as a categorical variable (none/non-formal schooling, primary, secondary, grade 12/higher). All results are based on 697 participants (605 in Meyerton and 92 in Ethembalethu) with the following exception: excludes 2 participants from Meyerton with missing BMI data in models with adjustment for the respective variable.

[c] Evaluates the current state of anxiety, how respondents "feel right now, that is, at this moment"; STAI questionnaire items 1–20.

[d] Evaluates how respondents "generally feel"; STAI questionnaire items 21–40.

between the Mn-exposed and non-exposed communities. The reasons for the strong association between depression and community, but the much weaker association between anxiety and community in our study compared to the study in the U.S., are not clear. One possibility is that the population in our study was demographically different from the Ohio study. Another explanation could be the different methodologies for assessing mood. The assessment of depression severity as a continuous measure likely minimized outcome measurement error, which might otherwise attenuate the associations. While we also assessed anxiety as a continuous measure in our validation sample, we observed that the Cronbach's alpha was higher for the BDI than for the STAI, especially for the most common home languages of the participants in our research sample. The lower internal consistency of the STAI likely resulted in greater outcome measurement error in assessing anxiety than in assessing depression. Further, consistent with this hypothesis, Mn-mood associations were reported more commonly among participants with higher educational attainment; this effect was more pronounced for anxiety than for depression. If individuals with lower education had difficulty reading or understanding the STAI, then we might have been less able to detect an association for anxiety in those with lower levels of educational attainment than in those with higher levels of education. The results of both our main and validity analyses are consistent with this hypothesis, although another possibility is that those achieving higher education levels respond to sensitive personal questions differently than those with less education.

Alternatively, assuming that there are direct relationships between Mn and mood, the dissociation of strength of the associations for depression vs. anxiety may indicate selectivity to brain pathways responsible for mood dysfunction. Our previous studies in workers exposed to Mn demonstrated dysfunction of the dopaminergic system as manifested by Mn-dose dependent upregulation of dopamine type 2 (D2)-receptors in the substantia nigra (Criswell et al., 2018a), differential binding of the vesicular monoamine transporter 2 radioligand [11C]dihydrotetrabenazine (DTBZ) in the thalamus and globus pallidus (Criswell et al., 2020), and lower dopa decarboxylase binding of the radioliagand 6-[18 F]fluoro-L-DOPA (FDOPA) in the caudate (Criswell et al., 2018b). These studies demonstrate the importance of the dopaminergic system in Mn neurotoxicity. We speculate that these same systems may contribute to the difference in depression (in particular) between the two communities. Animal models of stress-induced depression implicate mesolimbic dopamine pathways, and these effects may be mediated by brain-derived neurotrophic factor (Berton et al., 2006; Chaudhury et al., 2013). Even if these pathways are ultimately implicated in the pathogenesis of Mn-associated depression, we acknowledge that it is difficult to separate direct neurotoxic effects and indirect effects of stress related to environmental contamination.

Our study was conducted in the context of decades of high national crime and unemployment. We were able to observe differences between communities for depression, in particular, despite the high "basal" level of depression and anxiety, which are likely due, in part, to the lingering effects of Apartheid. While we chose a reference community that was demographically very similar to the Mn-exposed community, as well as with regard to land use and stressors such as noise, the communities are inherently different due to the Mn exposure itself (Racette et al., 2021). Nevertheless, the psychological impact of living in a community enduring generations of environmental pollution may be as important as the environmental toxin itself. As such, it is possible that, in this community, Mn pollution may serve as an environmental stressor. Whether Mn is causally related to depression through direct neurotoxic injury to mood-related basal ganglia pathways or indirectly related through the despair of living with a major source of air pollution proximate the settlements, the public health impact of this depression may include substance abuse, crime, and/or various forms of violent activities.

As with any study, there were some limitations. First, this was a cross-sectional study and it is therefore possible that some of the reported symptoms of depression and anxiety preceded residence in the respective communities. One of the depression subscores that was higher in the Mn-exposed than the reference community referred to "past" failures. Nevertheless, all of the subscores appeared to contribute to the association between Mn exposure and depression. Moreover, we observed greater differences in anxiety "state" symptoms than anxiety "trait" symptoms, indicating that these differences were more likely due to the way participants felt at the time of assessment, than longer term. We hypothesize that this difference in findings might be consistent with an environmental etiology, though we acknowledge that the relevant exposure period might differ between "state" and "trait" anxiety and is unknown. Second, we present only mean community exposures to Mn. The ongoing efforts to model individual-level inhalational Mn exposures from the smelter and other sources of airborne Mn should provide further insight into the association between Mn and mood, including dose-response relationships. Nonetheless, the differences in airborne Mn between the two communities was considerable, and it is likely that they were greater in the past, making our conclusions more plausible. Third, depression and anxiety are highly dependent on other life circumstances, including social determinants of health. To that end, we focused our study on two Black African communities consisting entirely of similar, low-income residents, and we performed multiple analyses to adjust for potential confounders. While some of these adjustments attenuated the associations somewhat, the overall findings – for depression in particular – were robust. Regardless, it is possible that there are additional cultural factors, related to social class, financial stress, etc., which we did not include in our analysis, though the municipalities within which these communities reside are largely comparable at the household and resident levels (Racette et al., 2021). Fourth, we selected participants using a population-based sampling method and found that residents of both communities were generally supportive of the research. Since not everyone agreed to participate, it is possible that those with greater depression and anxiety may have self-selected to participate, differentially (more often) in Meyerton. We have no evidence to suggest that this is true, however, and we conducted mood assessments in central locations within each community. Finally, we focused on a specific Mn-exposed community, so we do not know the extent to which these results are generalizable to other similar communities with environmental exposure to Mn.

5. Conclusion

We observed an association between mood and residential exposure to Mn at concentrations several orders of magnitude lower than reported in previous occupational studies that demonstrated similar associations. Our findings suggest that environmental Mn exposures may have implications beyond affecting motor system pathways. Future studies will address the public health impacts of these findings.

CRediT authorship contribution statement

Brad A. Racette, MD: Obtained funding, contributed to data collection, supervised the analyses, and wrote the first draft of this manuscript. **Gill Nelson, PhD:** Assisted with obtaining funding, contributed to data collection, and provided critical edits of this manuscript. **Wendy W. Dlamini, MSc:** Contributed to data collection, performed statistical analyses, and provided critical edits of this manuscript. **Tamara Hershey, PhD:** Contributed to data collection by developing the neuropsychological battery used in this study, supervised the analysis of the mood data, and provided critical edits of this manuscript. **Pradeep Prathibha, BS:** Assisted with data collection and curation and provided critical edits of this manuscript. **Jay R. Turner, DSc:** Assisted with obtaining funding, contributed to data collection, supervised the environmental monitoring, and provided critical edits of this manuscript. **Harvey Checkoway, PhD:** Assisted with obtaining funding and provided critical edits of this manuscript. **Lianne Sheppard, PhD:** assisted with obtaining funding, oversaw statistical analyses, and provided critical edits of this manuscript. **Susan Searles Nielsen, PhD:** Assisted with obtaining funding, oversaw data curation and statistical analyses, and provided critical edits to this manuscript.

Data statement

Data from research participants in this study, who authorized sharing of their research data, will be made available to investigators with appropriate expertise and research support, after publication of the primary aims of this study. All shared data will be de-identified and will be released in accordance with U.S. and South African regulations.

Declaration of Competing Interest

B.A. Racette receives research support from the following government and non-governmental organizations: National Institute of Environmental Health Sciences (NIEHS) (R01ES026891, R01ES026891-S1, R01ES025991, R01ES025991-02S1, R01ES030937-S1,R01ES029524), National Institute of Occupational Safety and Health (NIOSH) (R01OH011661), Cure Alzheimer's Fund, Department of Defense (PD190057), Hope Center for Neurologic Disorders (Washington University). Dr. Racette has received honoraria (personal compensation) for lectures from the University of Michigan and Harvard University. He has received personal compensation for peer review from the Parkinson Study Group, service on the National Advisory Environmental Health Sciences Council for NIEHS, and legal testimony on behalf of the Johnson and Bell law firm.

G. Nelson receives research support from the following government organization: NIEHS (R01ES026891, R01ES026891-S1, R01ES025991, R01ES025991-02S1). She also receives personal compensation from the journal "Occupational Health Southern Africa" for which she serves as editor.

W. W. Dlamini declares no disclosures relevant to the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.neuro.2021.05.017.

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