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DIFFERENCES IN PRENATAL EXPOSURE TO MANGANESE IN RURAL AND URBAN SOUTH AFRICAN COASTAL COMMUNITIES

Halina B. Röllin, Tahira Kootbodien, Jon Ø. Odland, Penny Theodorou

The contribution of diet to blood manganese concentrations in coastal rural and urban pregnant women in South Africa is explored.

Environmental Impact Statement

In African continent, including South Africa, there is a paucity of research on prenatal exposure to manganese, health outcomes and risk factors. This study investigates manganese levels at the time of delivery in two cohorts of pregnant women residing in rural and urban coastal settings of South Africa and examines birth outcomes and environmental factors that could influence manganese levels in the study population. Significant prenatal exposure to manganese was observed among rural and urban coastal South African communities. Indications are that coastal rural populations in South Africa are at greater risk of manganese exposure during pregnancy. Study highlighted the need to investigate possible co-exposure to a mixture of toxicants, e.g. lead.

Prenatal exposure to manganese in South African coastal communities

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ABSTRACT

Exposure to environmental sources and altered physiological processes of manganese uptake during pregnancy and its possible effect on prenatal and postnatal development are of concern. This study investigates manganese blood levels at the time of delivery across four cohorts of pregnant women residing in coastal communities of South Africa and examines birth outcomes and environmental factors that could influence manganese levels in the study population. The geometric mean (GM) manganese blood levels (MnB) for all women at delivery was 15.2 µg/L. Collectively, rural women reported higher MnB concentrations (GM, 16.1 µg/L) than urban women (GM, 13.5 µg/L, $p<0.001$). Of the 302 cord blood samples drawn from the study participants (rural women only), GM MnB levels reported for three rural sites were 25.8 µg/L (Rural 1), 33.4 µg/L (Rural 2) and 43.0 µg/L (Rural 3) and were twice as high as their respective maternal levels. However, no significant correlations were found between maternal and cord MnB levels across the 3 study areas. Factors associated with elevated maternal MnB levels, after adjusting for gestational age were: women living in a rural area (Rural 2) ($p = 0.021$); women drinking potable water from an outdoor/communal tap sourced from municipality ($p = 0.021$); drinking water from river/stream ($p = 0.036$); younger maternal age ($p = 0.026$); consuming leafy vegetables once a week ($p = 0.034$); and elevated maternal blood lead concentrations (PbB) ($p = 0.002$). The results indicate that a MnB concentration in rural women during pregnancy is higher compared to urban women and increases with manganese intake from food and water.

Keywords: manganese, prenatal exposure, diet, water source, lead

1. Introduction

Manganese (Mn) is an essential trace element required for a variety of biological processes, with human body maintaining stable tissue levels of Mn through strict homeostatic control of both absorption and excretion [1,2]. As an essential metal, Mn is involved in the formation of bone, and in the metabolism of amino acids, cholesterol and carbohydrates. Mn is required in various metalloenzymes including arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and superoxide dismutase [SOD - the principal antioxidant in mitochondria] [1, 3]. Furthermore, Mn assists in the maintenance of healthy reproductive, nervous and immune systems, and is involved in blood sugar regulation, blood clotting and the formation of cartilage and lubricating fluid in the joints [4].

The brain is a critical target organ for Mn deposition as Mn crosses the blood-brain barrier (BBB), and this is the first step in the pathogenesis of the neurotoxicity of Mn. In occupational settings, excessive exposure to Mn via inhalation has been shown to have detrimental effects on the lungs and to accumulate in the brain, causing irreversible brain disease, to some extent similar to Parkinson's disease (PD) [5-7].

It has been shown that pregnant women, foetuses, neonates and young children are known to retain Mn to a greater extent than the general adult population. In *utero*, foetal uptake of Mn is influenced by maternal levels and by active placental transport of Mn from the mother to foetus. It is understood that Mn absorption during pregnancy is greatly influenced by maternal iron status as iron deficiency increases absorption of Mn due to the divalent metal transporter 1 (DMT1), the primary non-heme iron transporter in the intestine, which transports not only iron but also Mn and other trace elements. Women with low iron stores absorb about 5% of dietary Mn versus 1% in women with normal iron levels [8]. It is of concern, given the fact that iron deficiency anaemia during pregnancy is common worldwide, particularly in less developed areas of the world [9]. Very few studies have examined the effects of maternal Mn levels during pregnancy on birth outcomes. It has been shown that due to the active transport of Mn across the placenta, levels of Mn in cord blood are higher than those in the maternal blood at delivery [10-13]. Su et al. conducted a study in children from the general population in Tapei, Taiwan and have found an association between in *utero* exposure to Mn and the fine-motor developmental quotients in infants when they reached age of six months. Their mean cord blood manganese levels were 51.9 ug/L [14]. A study by Vigeh et al., reported on the relationship between maternal blood Mn concentration and intrauterine growth restriction (IUGR) [4]. An inverted U-shape relationship between maternal blood Mn levels and infant birth weight has been shown by other [15]. A number of studies on neonates and infants have confirmed decreased elimination mechanisms for Mn, making neonates and infants receiving total parenteral nutrition or formulas containing Mn highly susceptible to Mn neurotoxicity [16-19]. A significant increase in blood Mn concentrations with increasing gestational age and postpartum has been shown [20-23].

In addition, environmental exposure to Mn through inhalation and ingestion continue in early childhood and may cause neurobehavioral deficits in children. For example, studies in Bangladesh suggest that ingesting high doses of Mn in drinking water is associated with neurotoxic effects in children, interfering with their intellectual function, as well as causing infant mortality [24,25]. Similar studies have shown that drinking water containing much lower concentrations of Mn than those found in Bangladesh also caused intellectual impairment in school children [26]. The environmental exposure to Mn from industrial and mining sources, and negative cognitive performance of children were also reported [27,28] . While these studies report deficits in children, the contribution of pre-natal exposure to excess Mn is unknown.

In the African continent, including South Africa, there is a paucity of research on prenatal exposure to manganese, health outcomes and risk factors. The present study reports on the levels of Mn in blood at delivery in cohorts of rural and urban women residing along coastal regions of South Africa and examines its association with birth outcomes, socioeconomic, diet and environmental factors.

This study is a part of on-going multidisciplinary, multi-institutional research collaboration between South Africa and Norway that evaluates exposures and effects of persistent toxic substances (PTS) on reproductive health and birth outcomes in populations of the northern and southern hemispheres.

2. Materials and methods

2.1.Study sites and participants

Four study sites were selected (Figure 1). The three rural sites (Rural 1, Rural 2 and Rural 3) were situated along the Indian Ocean coast of the KwaZulu Natal (KZN) Province, and one urban site (Cape Town) was situated along the Atlantic Ocean coast of the Western Cape Province. Rural 1 is situated in the far northern region of KZN, which borders with Mozambique. Mainly subsistence farming and controlled fishing takes place at this site, and its geographical location exposes it to mining, industrial, farming and vehicular activities in South Africa and neighbouring countries. Rural 2 is surrounded mainly by small and commercial farming activities. Rural 3 is situated within a commercial farming region (mainly sugar cane activities) but in the vicinity of industrial sites and activities such as aluminium smelting, coal terminals and ports. Rural 2 and Rural 3 are situated 400 km and 100 km south of Rural 1, respectively. The urban site is the city of Cape Town which is surrounded by commercial, industrial and port activities.

Potential study candidates were recruited by a health worker on duty, from women who were admitted for delivery at the local hospitals. A trained research assistant briefly explained the objectives of the study and distributed a detailed information sheet about the project. All women who agreed to participate signed an informed consent form and agreed to donate blood before delivery and cord-blood samples post-partum. In addition, the study participants agreed to answer a socio-demographic questionnaire which included the topics of diet, lifestyle, health status and demographic factors, and consented to access and use of the birth outcomes data obtained from the hospitals, for research purposes. A design of socio-demographic questionnaire was not specific for Mn exposure but for exposure to environmental pollutants in general. The dietary part of the questionnaire recorded intake frequency of various basic foods during pregnancy. After delivery, records from hospital files were extracted, including maternal and newborns characteristics such as weight, length and head circumference, gestational age and birth complications. In total, 550 delivering women participated in the study.

2.2.Sampling procedure

From each woman, a volume of 10 ml of venous blood was collected into BD Vacutainer tube (10 ml capacity and containing EDTA) before delivery, and 10 ml of umbilical cord blood were collected post-partum. Cord blood Mn levels were available for the rural cohort only (n=302).

2.3.Processing and analytical procedure

The analyses for Mn content in whole blood and cord blood were performed using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) instrument (Agilent 7500ce ICP-MS with an Octopole Reaction System). Contamination-free vessels and procedures were used throughout and validation of results was accomplished by including certified standards in the analyses.

Briefly, the whole blood samples (0.5ml volumes) were digested with nitric acid (1ml) at 90^{o}C for 2 hours. After cooling, the internal standard ^{204}Tl was added and samples were diluted to a final volume of 7 ml. To eliminate any mass interference for ^{55}Mn, ^{72}Ge was used as an internal standard, and the analysis was performed in Helium acquisition mode. For quality assurance, two certified reference controls, viz. Seronorm ^{TM}Trace Elements (Sero LTD., Billingstad, Norway) in whole blood, (Levels 1 and 2) were analysed for Mn in every analytical run, in intervals of 10 samples. Aliquots of each sample were analysed in triplicate. The detection limits (three times the standard deviation of all blank samples) for Mn was 0.07µg/L and for Pb 0.04µg/L.

2.4.Statistical analyses

The goal of the analysis was to determine differences in maternal blood Mn concentrations between the rural and urban groups and to account for dietary and selected environmental factors attributing to these differences. All maternal blood Mn measurements were converted to their natural logarithmic values in order to normalise the positively skewed blood Mn levels. Normally distributed variables were presented as mean and standard deviation (± SD). Low birth weight was defined as a birth weight of less than 2500g. Statistical analyses using the chi-square test and Kruskal-Wallis rank test were performed as appropriate across the study sites. The relationships between cord MnB and maternal MnB levels, cord PbB and maternal PbB levels and maternal MnB and maternal PbB levels were examined using Spearman correlation coefficients.

To assess variables or factors that were significantly associated with maternal blood Mn levels, univariate and bivariate regression analyses were performed on the demographic, diet and lifestyle, environmental and birth outcome variables against log-transformed Mn blood levels. These variables were included *a priori* and were based on biological plausibility. Covariates were added, one at a time, to the model that were associated with MnB levels in the bivariate models (P<0.10), thereby minimising collinearity. For model building we used all eligible study participants with complete maternal blood manganese concentrations at delivery excluding outliers were used (n=545). In addition, we excluded participants with missing data on covariates in the final model. All statistical analyses were performed using Stata version 12 statistical software [29].

2.5.Ethical considerations

Ethics approval for the study was obtained from the Human Research Ethics Committee of the University of Witwatersrand in Johannesburg (Protocol no. M10742). Confidentiality was maintained by assigning participants identification numbers. During informed consent process, it was emphasised that participation was voluntary and could be withdrawn at any time.

3. Results

3.1.Population characteristics

The main population characteristics and obstetric and newborn parameters, by residential area (Rural 1, Rural 2, Rural 3 and Urban) are presented in Table 1. Most of the characteristics among the four groups were significantly different. Three hundred and fifty women (64%) lived in a rural area, and 200 (36%) in an urban area. Collectively among the rural participants, women had a mean age of 24.5 ± 6.3 years; were mostly single (73%) and more than a third had reached tertiary education (38%). A large majority of participants were unemployed (86%), lived in formal housing (83%) and owned their homes (94%). Wood (32%) and electricity (57%) were fuels used often for cooking. Rural mothers sourced their potable drinking water predominantly from a communal outside tap (71%), however untreated borehole water (10%) and water from a river or stream (9%) was also used. There were no significant differences between the socio-economic characteristics of women living in Rural groups 1, 2 and 3.

Urban participants were slightly older (mean age: 26.1 ± 6.3 years) and more than half were married or living together (58%). Most of the participants (91%) had reached secondary level of education, and tertiary education was minimal (only 7.6%). Sixty two percent were unemployed and less than a third (31%) rented their home. More than half of the participants lived in formal housing (57%) and 21% lived in informal housing. Electricity (97%) was the predominant fuel used for cooking. Potable drinking water was sourced from both outdoor (67%) and indoor (33%) taps only, with no water being sourced from rivers or streams.

The mean (± SD) distance to the nearest highway from rural homes collectively was 3.3 (6.9) km compared to 0.8 (1.3) km from urban homes. A large majority of women (74%) living in Rural 3 and 48% of women living in Urban area perceived the air quality to be bad in their neighbourhood. Similarly women living in Rural 3 (58%) and Urban area (49%) perceived the air quality in their neighbourhood to be poor.

Urban participants were more exposed to passive smoking in their households, compared with collective rural participants (48% versus 32%, Chi-square p-value < 0.001).

3.2.Obstetric and newborns parameters

Obstetric and newborns parameters are indicated in Table 1. In the total study population, half of the mothers were primiparous (54%). The mean (± SD) gestational age at delivery was 38.1 (2.2) weeks. The mean (± SD) pre-delivery maternal weight was 74.3 (15.7) kg. Mean (± SD) birth weight was 3063.5 (525) g, ranging from 855 to 5150 g. The proportion of newborns with low birth weight was 11%. Mean (± SD) birth length was 49.5 (3.6) cm, ranging from 31 to 66 cm. Mean (± SD) head circumference was 34.9 (2.1) cm, ranging from 25 to 50 cm. The proportion of male live births in the study population was 53%. There were significant differences in gestational age (Kruskal-Wallis rank test, p = 0.001), maternal weight (Kruskal-Wallis rank test, p = 0.008) and birth weight (Kruskal-Wallis rank test, p = 0.0001) among women across the three rural groups. When comparing all four sites, there were significant differences in gestational age (Kruskal-Wallis rank test, p < 0.001), maternal weight (Kruskal-Wallis rank test, p = 0.025), birth weight (Kruskal-Wallis rank test, p = 0.002) and birth length (Kruskal-Wallis rank test, p = 0.009).

3.3. Levels of manganese and lead in maternal blood and manganese levels in cord blood

Overall, GM MnB level for women at delivery was 15.2 µg/L (Table 2). In the collective rural group, MnB levels were highest among women living in Rural 2 (GM, 17.3 µg/L), followed by Rural 3 (GM, 15.7 µg/L) and Rural 1 (GM, 14.1 µg/L) respectively. Urban women had the lowest MnB levels (13.5 µg/L) (Kruskal-Wallis rank test, p = 0.001).

Of the 302 cord blood samples, the GM cord MnB levels from Rural 1, Rural 2 and Rural 3 groups were 25.8 µg/L, 33.4 µg/L and 43.0 µg/L respectively. Among the three groups, no significant linear associations were found between maternal MnB and cord MnB levels (Rural 1, Spearman rho=0.04, p=0.687; Rural 2, Spearman rho=0.05, p=0.464; Rural 3, Spearman rho=0.16, p=0.318).

Maternal PbB levels were highest among women in Rural 1 (GM, 2.4 µg/dL), followed by Rural 3 and 2 respectively (GM, 1.6 µg/dL and GM, 1.5 µg/dL). Urban women reported the lowest PbB levels (GM, 1.0 µg/dL) (Kruskal-Wallis rank test, p=0.001). Cord PbB levels were highest among women living in Rural 1 (GM, 1.7 µg/dL) followed by Rural 2 and 3 respectively (GM, 1.2 µg/dL, GM,1.1 µg/dL). Strong significant linear associations were found between maternal PbB and cord PbB levels across women living in Rural 1 (Spearman rho=0.88, p<0.001), Rural 2 (Spearman rho=0.65, p<0.001) and Rural 3 (Spearman rho=0.76, p<0.001) respectively.

When examining the relationship between maternal MnB and PbB levels at delivery, a moderate linear association was found in women living in Rural 1 (Spearman rho=0.36, p=0.003) and weak linear association was found in women living in Rural 2 (Spearman rho=0.15, p=0.03). No significant linear association was found between maternal MnB and PbB levels in Rural 3 (Spearman rho=0.22, p=0.121) and urban cohort

(Spearman rho=0.08, p=0.263). Twenty five women (4.6%) reported PbB levels above the 5 µg/dL reference level: 12% (12/100) in Rural 1, 3.5% (7/200) in Rural 2, 6% (3/50) in Rural 3 and 1.5% in Urban (3/195) groups respectively.

3.4. Factors associated with maternal levels of manganese

Table 3 shows the geometric mean (GM) of MnB levels presented in relation to selected environmental and dietary characteristics for the study participants. MnB was not associated with socio-economic or other family characteristics such as marital status and maternal education (data not shown). Women living in Rural 2 ($p < 0.001$) and 3 ($p = 0.015$) were more likely to have elevated maternal MnB levels when compared to urban women. Mothers who lived further away from the highway (i.e. more than 1200 m) had higher levels of MnB (GM, 15.84 µg/L, $p = 0.009$), compared with mothers who lived less than 200 m from a highway (GM, 13.22 µg/L). Higher MnB levels were found in women who consumed leafy vegetables once a week (GM, 16.40 µg/L, $p = 0.022$), compared with maternal MnB levels in women who seldom ate leafy vegetables (GM, 12.95 µg/L). MnB levels were found to be lower among women who applied pesticides in their garden (GM, 14.24 versus 15.46 µg/L, $p = 0.042$) and who consumed meat once a week (GM, 14.60 µg/L, $p = 0.007$), versus women who seldom ate meat (GM, 16.64 µg/L).

As shown in Figure 2, maternal MnB was higher among women who sourced their drinking potable water from a communal outdoor tap (GM, 15.91 µg/L, $p < 0.001$) and river or stream (GM, 17.08 µg/L, $p = 0.006$), compared with potable water from an indoor tap (GM, 13.71 µg/L).

There was no association between maternal MnB levels and the following birth outcome variables (Table 4): maternal weight ($p = 0.318$), low birth weight ($p = 0.474$), birth length ($p = 0.654$), head circumference ($p = 0.372$), gender ($p = 0.873$) or parity ($p = 0.386$). However, lower MnB levels were associated with gestational age at the 10% level of significance ($p = 0.071$).

In the final multivariate model (Table 5), after adjusting for gestational age, women living in Rural 2 were more likely to have higher MnB levels than women living in other study sites ($p = 0.021$). Elevated MnB was significantly associated with PbB levels ($p=0.002$); sourcing potable drinking water from a communal outdoor tap ($p = 0.021$); sourcing drinking water from river or stream ($p = 0.036$); younger maternal age ($p = 0.026$), and consuming leafy vegetables once a week ($p = 0.034$). Consuming meat once a week was associated with lower MnB levels ($p = 0.016$).

4. Discussion

Overall, the present study found MnB concentrations in South African women at delivery to be comparable with MnB levels in similar populations residing in industrial settings of Canada (GM 14.6 µg/L), Australia (mean 13 µg/L), Sweden (GM 12 µg/L) and Norway (GM 10.7 µg/L) [30,22,23,20]. However, these concentrations were much lower than those reported in comparable studies from China (mean 55 µg/L) and

France (mean 23 μg/L) [31,32]. Mn levels at different stages of pregnancy were not reported in the current study. However, studies have shown that Mn levels are found to be at their highest at delivery and postpartum, and are an indication of prenatal exposure [20,30].

The current study found regional differences in MnB concentrations with significantly higher mean MnB levels in rural women compared to urban women. In the rural study cohort, Mn was also measured in the respective umbilical cord blood samples, and the Mn levels in cord bloods were found to be double those found in the respective maternal samples. These findings are in agreement with the outcomes of the pilot of this study and other investigations, except that no significant correlation was found between maternal and the respective cord blood Mn levels, as has been reported in other studies [12,30]. Some studies have found some correlation between maternal MnB levels and birth outcomes such as birth weight and head circumference; however, these correlations were not evident in the current study cohort [15.31].

Thus, this study found that rural woman, residing further away from busy roads, had higher MnB levels, when compared with urban participants who lived very close to major roads. A possible explanation for this finding may be major differences in climatic conditions in the rural study areas (subtropical and situated along the Indian Ocean), compared to the urban study area (cooler and situated along the Atlantic Ocean); these climatic differences may influence the atmospheric transport and dispersion rates for Mn and other contaminants. The dispersion of the inland (Highveld) air pollution towards the Indian Ocean may be an additional contributor. The findings of the current study are in agreement with the previous study, where MnB above the limit set by the Agency for Toxic Substances and Disease Registry (ATSDR) were found in 4.2% of children in Cape Town and 12.5% of children in the city of Johannesburg [33-35].

It is evident from the current investigation that other environmental and dietary factors may influence bioavailability of Mn during pregnancy. According to others research reports, higher MnB levels were found in women residing close to agricultural areas where pesticides are regularly sprayed [21, 30, 36]. In contrast, the present study found lower MnB concentrations in women who reported using pesticides in their gardens and resided close to agricultural areas. This may be due to differences in the types of pesticides used in South Africa which may not contain Mn. This study also found that exposure to passive smoking in the household increased MnB concentrations, although this was not confirmed in the multivariate model. As far as diet is concerned, this study found that consuming leafy vegetables at least once a week increased MnB levels, suggesting an affinity for Mn uptake from the soil, by leafy vegetables. Consumption of vine and root vegetables did not influence Mn concentrations in this study. Interestingly, consumption of meat once a week showed a negative association, suggesting that iron intake may have a protective effect in terms of Mn absorption. The study performed on the general population by Baldwin et al reported that leafy vegetables contributed positively to MnB levels [37]. Fifty percent of women who participated in this study indicated that they consumed meat once a week and 30% indicated daily consumption of meat which may be protective against anaemia (Table 3). Tholin et al found no relationship between MnB and iron status in healthy pregnant Swedish women [23]. Baldwin et al also reported serum iron being negatively related to MnB levels in general population [37]. These findings however cannot be compared in this study as iron status was not measured.

The most important contributing factor to MnB levels in this study population was the source of the ingested water. South Africa is a country with sparse water resources and far distances between water sources and human settlements. These factors, in addition to the economic constraints, particularly in the rural and informal areas, have resulted in potable water supply not being piped to individual households. Instead, potable water (chemically purified by municipal water treatment plants and certified for human consumption) is frequently supplied to communal outdoor taps, where water is collected by the community members (mostly women) into various vessels and stored in the individual households. In this study population, women who reported having access to drinking potable water from taps inside individual households (via a municipal water piping system) had the lowest MnB levels (13.71 µg/L). Women using communal outdoor taps supplying the same water as their source of drinking water had significantly higher MnB levels, followed by women using river or stream water. This suggests that the storage of potable water at household level in rural settings in South Africa may be an important contributor of exposure to Mn and other contaminants, and warrants further investigation. Ideally, populations which accesses potable water from communal facilities should be made aware and educated about the health risks of using unsuitable containers for water collection and storage.

In the final multivariate analysis, the source of drinking water; consumption of leafy vegetables once a week; and higher PbB levels and younger maternal age were all positively associated with increased MnB concentrations. On the other hand, consumption of meat once a week showed a negative association, possibly due to the potentially protective effect of iron uptake. The positive association between MnB and PbB concentrations in the population of this study was found and may suggest same sources for women living in Rural 1 and Rural 2. Recent studies have shown evidence of synergism between Pb and Mn in early childhood development at 12 and 24 months, and impacts on full-scale and verbal IQ among school-age children, which was not examined in the present study [38,39]. This synergism between Mn and Pb warrants further investigation due to its associated public health implications.

Some of the limitations identified for this study are the potential discrepancies in self-reported data on diet and consumption frequency, as well as perceptions around air quality and environmental pollution.

5. Conclusion

This study found regional differences in concentration of manganese at delivery in South African communities, and characterised environmental and dietary contributors. It also highlighted the need to investigate possible co-exposure to a mixture of toxicants (e.g. Mn and Pb) in prenatal and postnatal stages when assessing subsequent childhood development.

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Figure 1 Map of South Africa depicting urban and rural study sites

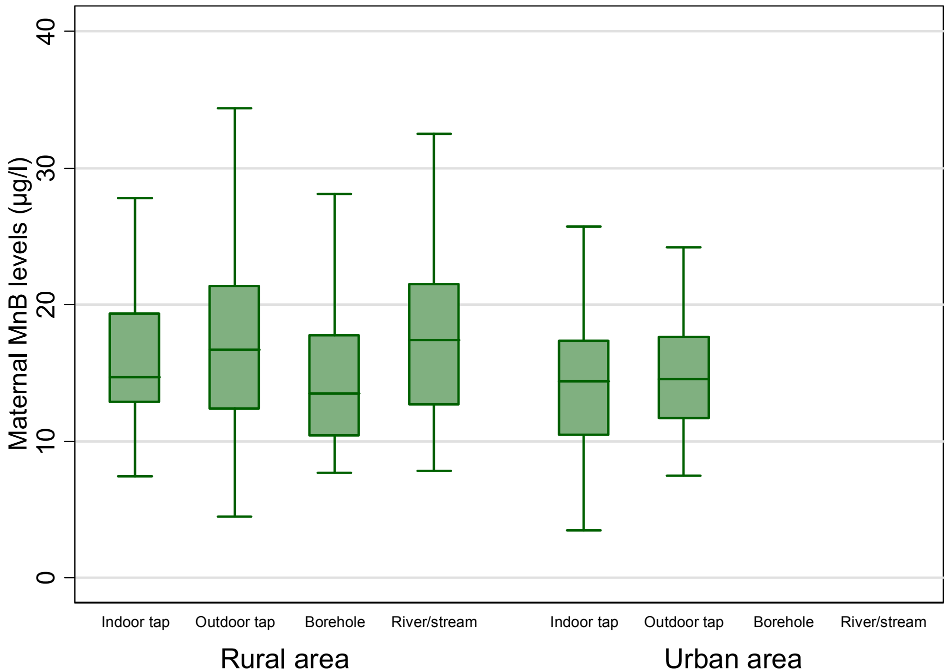

Figure 2 Maternal MnB levels in relation to reported source of drinking water supply (observations outside the 95% CI are not shown)

Table 1 Population characteristics, obstetric and newborn parameters by residential area

Characteristic	Total (n=550)	Rural total (n=350)	Rural 1 (n=100)	Rural 2 (n=200)	Rural 3 (n=50)	Urban (n=200)	p-value
Mother's characteristics							
Age (years) (mean, SD)	25.1 (6.4)	24.5 (6.3)	23.8 (6.8)	24.6 (6.0)	25.6 (6.4)	26.1 (6.3)	0.003
Marital status (n, %)							
Married / living together	205 (38.0)	91 (26.6)	37 (37.0)	47 (24.5)	7 (14.0)	114 (57.9)	
Single	330 (61.2)	249 (72.8)	63 (63.0)	143 (74.5)	43 (86.0)	81 (41.1)	
Divorced / widowed	4 (0.8)	2 (0.6)	0 (0)	2 (1.0)	0 (0)	2 (1.0)	<0.001
Education (n, %)							
None / Primary	52 (9.9)	50 (15.2)	23 (26.1)	23 (12.1)	4 (8.0)	2 (1.0)	
Secondary	334 (63.6)	154 (47.0)	31 (35.2)	103 (54.2)	20 (40.0)	180 (91.4)	
Tertiary	139 (26.5)	124 (37.8)	34 (38.4)	64 (33.7)	26 (52.0)	15 (7.6)	<0.001
Percentage unemployed (n, %)	416 (77.3)	294 (86.0)	94 (94.0)	156 (81.3)	44 (88.0)	122 (62.2)	<0.001
Ownership of home (n, %)							
Owned	459 (85.2)	324 (94.2)	100 (100)	180 (92.8)	44 (88.0)	135 (69.2)	
Rented	80 (14.8)	20 (5.8)	0 (0)	14 (7.2)	6 (12.0)	60 (30.8)	<0.001
Housing type (n, %)							
Formal housing	396 (73.6)	285 (83.3)	91 (91.0)	163 (84.5)	31 (63.3)	111 (56.6)	
Flat	32 (6.0)	12 (3.5)	0 (0)	12 (6.2)	0 (0)	20 (10.2)	
Backyard dwelling	21 (3.9)	3 (0.9)	0 (0)	2 (1.0)	1 (2.0)	18 (9.2)	
Informal housing	67 (12.5)	26 (7.6)	7 (7.0)	16 (8.3)	3 (6.1)	41 (20.9)	
Other	22 (4.0)	16 (4.7)	2 (2.0)	0 (0)	14 (28.6)	6 (3.1)	<0.001
Fuel use for cooking (n, %)							
Electricity	337 (71.5)	195 (56.7)	15 (15.0)	145 (74.5)	35 (70.0)	192 (97.5)	
Paraffin	29 (5.4)	26 (7.5)	0 (0)	24 (12.4)	2 (4.0)	3 (1.5)	
Gas	15 (2.8)	13 (3.8)	10 (10.0)	3 (1.6)	0 (0)	2 (1.0)	
Wood	110 (20.3)	110 (32.0)	75 (75.0)	22 (11.3)	13 (26.0)	0 (0)	<0.001
Source of drinking water (n, %)							
Indoor tap	168 (31.5)	36 (10.7)	3 (3.1)	23 (11.9)	10 (21.7)	132 (67.0)	
Outdoor tap	305 (57.1)	240 (71.2)	58 (59.8)	151 (77.8)	31 (67.4)	65 (33.0)	

Borehole	32 (6.0)	32 (9.5)	29 (29.9)	2 (1.0)	1 (2.2)	0 (0)	
River/stream	39 (5.4)	39 (8.6)	7 (7.2)	18 (9.3)	4 (8.7)	0 (0)	<0.001
Exposure to passive smoking in the household (n, %)							
No	331 (62.0)	230 (67.9)	81 (81.0)	115 (60.5)	34 (68.0)	101 (51.8)	
Yes	203 (38.0)	109 (32.2)	19 (19.0)	75 (39.5)	16(32.0)	94 (48.2)	<0.001
Distance to nearest highway (km) (mean, SD)	2.4 (5.7)	3.3 (6.9)	1.1 (0.4)	4.0 (7.6)	5.1 (9.7)	0.8 (1.3)	<0.001
Perception that air quality is bad in the neighbourhood (n, %)	130 (24.1)	37 (10.8)	0 (0)	5(2.7)	32 (74.4)	93 (48.2)	<0.001
Perception of environmental pollution around the home (n, %)	129 (24.4)	38 (11.1)	0 (0)	9 (4.7)	29 (58.0)	91 (48.9)	<0.001
Obstetric and newborn parameters							
Gestational age (mean, SD)	38.1 (2.2)	37.8 (1.7)	38.2 (1.8)	37.4 (1.6)	38.8 (1.4)	38.7 (2.9)	<0.001
Maternal weight (kg) (mean, SD)	74.3 (15.7)	73.2 (12.7)	70.1 (11.2)	74.2 (12.9)	75.3 (13.6)	76.5 (20.1)	0.025
Birth weight (g) (mean, SD)	3063.5 (525)	3053.3 (494.3)	3152.8 (463.5)	2956.9 (504.9)	3246.2 (415.9)	3080.9 (576.6)	0.002
Birth length (cm) (mean, SD)	49.5 (3.6)	49.2 (3.1)	49.0 (3.0)	49 (3.3)	50.1 (2.1)	50.0 (4.2)	0.009
Head circumference (cm) (mean, SD)	34.9 (2.1)	35.1 (1.8)	35.1 (1.3)	34.9 (2.0)	35.2 (1.5)	34.6 (2.6)	0.055
Sex (% boys)	53	51	51.7	50.3	52	58	0.461
Parity (n, %)							
0	286 (54.0)	173 (50.9)	43 (44.3)	101 (52.3)	29 (58.0)	113 (59.8)	
1+	243 (46.0)	167 (49.1)	54 (55.7)	92 (47.7)	21 (42.0)	76 (40.2)	0.081

Table 2 Maternal blood manganese (MnB) and blood lead (PbB) levels at delivery (total and by site)

	Total (n=545)	Rural total (n=350)	Rural 1 (n=100)	Rural 2 (n=200)	Rural 3 (n=50)	Urban (n=195)
Maternal MnB levels (µg/L)						
Mean	16.3	17.4	15.3	18.5	16.9	14.5
SD	6.5	7.1	6.4	7.2	6.8	4.9
Geometric mean	15.2	16.1	14.1	17.3	15.7	13.5
95% CI	14.6 – 15.6	15.3 -16.7	13.0 – 15.2	16.4 – 18.2	14.1 – 17.6	12.8 – 14.3
Median	15.2	16.1	13.8	17.1	17.2	14.5
Range	2.4 – 43.9	1.5 – 43.9	4.5 – 40.3	7.2 – 43.9	6.1 – 38.8	2.4 – 28.9
IQR	12.1 -19.1	12.3 – 21.0	11.2 – 17.5	13.3 – 22.7	12.9 – 20.0	11.2 – 17.5
Maternal PbB levels (µg/dL)						
Mean	1.9	2.2	3.1	1.9	1.8	1.3
SD	2.0	2.2	3.5	1.3	1.3	1.1
Geometric mean	1.4	1.7	2.4	1.5	1.6	1.0
95% CI	1.3 – 1.5	1.6 – 1.9	2.1 – 2.7	1.4 – 1.7	1.3 – 1.8	0.9 – 1.1
Median	1.5	1.7	2.5	1.6	1.4	1.0
Range	0.04 – 31.7	0.04 – 31.7	0.3 – 31.7	0.04 – 10.7	0.7 – 6.4	0.5 – 6.2
IQR	0.1 – 12.0	1.9 – 2.6	1.5 – 3.5	1.1 – 2.3	1.1 – 2.1	0.5 – 1.6

Table 3 Maternal MnB levels (µg/L) by selected environmental and dietary characteristics of participant

Characteristic	frequency	%	MnB (GM)	β	95% CI	p-value
Residential area						
Urban	195	36	13.54	Ref		
Rural 1	100	18	14.10	0.04	-0.05 to 0.13	0.409
Rural 2	200	37	17.27	0.24	0.17 to 0.32	< 0.001
Rural 3	50	9	15.72	0.15	0.03 to 0.27	0.015
Fuel use for cooking						
Electricity	383	72	15.13	Ref		
Paraffin	29	5	16.07	0.06	-0.09 to 0.21	0.437
Gas	15	3	14.53	0.03	-0.18 to 0.23	0.806
Wood	110	20	14.79	-0.02	-0.11 to 0.06	0.596
Source of drinking water						
Indoor tap	168	32	13.71	Ref		
Outdoor tap	305	57	15.91	0.14	0.07 to 0.22	< 0.001
Borehole	32	6	14.01	0.02	-0.12 to 0.17	0.774
River/stream	29	5	17.08	0.21	0.06 to 0.38	0.006
Distance to nearest highway						
< 200 m	41	8	13.22	Ref		
200 – 1200 m	338	62	15.02	0.13	-0.01 to 0.26	0.053
> 1200 m	166	30	15.84	0.18	0.04 to 0.32	0.009
Exposure to passive smoking in the household						
No	330	62	14.81	Ref		
Yes	200	38	15.61	0.05	-0.02 to 0.12	0.149
Perception of air quality in area						
Good	393	75	15.23	Ref		
Bad	130	25	14.47	-0.05	-0.13 to 0.03	0.198
Perception of environmental pollution around the home						
No	398	76	15.42	Ref		
Yes	127	24	14.52	0.06	-0.02 to 0.14	0.140
Grow own fruit and vegetables						
No	303	57	14.84	Ref		
Yes	233	43	15.52	0.04	-0.02 to 0.11	0.198
Use of pesticides in the garden						
No	343	72	15.46	Ref		
Yes	137	28	14.24	-0.08	-0.16 to -0.01	0.042
Consumption of root vegetables						
seldom	10	2	14.27	Ref		
once / week	93	17	15.28	0.07	-0.19 to 0.33	0.610
everyday	447	81	15.11	0.06	-0.20 to 0.31	0.660
Consumption of leafy vegetables						
seldom	17	3	12.95	Ref		
once / week	117	22	16.44	0.24	0.03 to 0.44	0.022
everyday	398	75	14.81	0.13	-0.06 to 0.33	0.102
Consumption of vine vegetables						
seldom	26	5	14.81	Ref		
once / week	164	31	15.84	0.07	-0.10 to 0.23	0.424
everyday	341	64	13.57	0.01	-0.15 to 0.16	0.970
Consumption of meat						
seldom	95	20	16.64	Ref		
once / week	246	50	14.60	-0.13	-0.22 to -0.35	0.007
everyday	145	30	15.68	-0.06	-0.16 to 0.04	0.260

GM = geometric mean

Table 4 Maternal MnB levels (μg/L) by obstetric and neonatal outcomes

Characteristic	β	95% CI	p-value
Gestational age	-0.02	-0.03 to 0.001	0.071
Maternal weight	-0.001	-0.003 to 0.001	0.318
Birth weight			
≤ 2500 g	Reference		
> 2500 g	-0.03	0.01 to 0.06	0.474
Birth length	-0.002	-0.01 to 0.007	0.654
Head circumference	-0.007	-0.02 to 0.008	0.372
Gender			
Male	Reference		
Female	0.006	-0.06 to 0.07	0.873
Parity			
0	Reference		
1+	0.03	-0.04 to 0.09	0.386

Table 5 Characteristics predicating elevated log transformed MnB levels at delivery: multivariate analysis

Characteristic	β	95% CI	t	p-value
Residential area				
Urban	Reference			
Rural 1	-0.14	-0.28 to 0.01	-1.82	0.069
Rural 2	0.13	0.02 to 0.25	2.32	0.021
Rural 3	0.06	-0.09 to 0.20	0.76	0.450
Source of drinking water				
Indoor tap	Reference			
Outdoor tap	0.12	0.02 to 0.23	3.51	0.021
Borehole	0.10	-0.18 to 0.21	0.11	0.915
River/stream	0.26	-0.01 to 0.389	2.10	0.036
Consumption of leafy vegetables				
Seldom	Reference			
Once a week	0.23	0.02 to 0.45	2.12	0.034
Everyday	0.17	-0.04 to 0.37	1.61	0.109
Consumption of meat				
Seldom	Reference			
Once a week	-0.13	-0.24 to -0.08	-2.42	0.016
Everyday	-0.03	-0.14 to 0.41	-0.48	0.663
Blood Pb levels	0.01	0.002 to 0.006	3.13	0.002
Gestational age	-0.01	-0.02 to 0.01	-0.77	0.441
Maternal age	-0.01	-0.01 to -0.001	-2.24	0.026

$F_{(13, 389)} = 6.11$; $p < 0.001$; $R^2 = 0.1669$