

Chapter 5

Levels of Lead Across Pregnancy in Women from Major and Small Villages in the Central District, Botswana

5.1 Abstract

Blood lead (PbB) levels were measured in a prospective cohort study of pregnant women living in selected villages in the Central Administrative District of Botswana to characterize PbB changes during pregnancy and investigate if PbB levels differed by location. PbB were measured at weeks 8-12, 20-24 and 34-36 respectively among women aged 18-44 years. A total of 137, 126 and 106 women were enrolled at the first, second and third trimesters respectively from four locations of varying socioeconomic and environmental status. PbB concentrations ranged from 0.5-12.90 $\mu\text{g}/\text{dL}$ with an overall mean ($\pm\text{SEM}$) of 2.34(± 0.098) $\mu\text{g}/\text{dL}$. PbB concentrations of $\geq 5\mu\text{g}/\text{dL}$ were observed in 5.5%, 5.6% and 3.1% during the first, second and third trimesters of pregnancy. A significant increase in PbB levels was observed between the first and third trimester ($p=0.01$). Mean PbB ($\pm\text{SEM}$) for the first, second and third trimesters were 1.96(± 0.14) $\mu\text{g}/\text{dL}$, 2.49(± 0.17) $\mu\text{g}/\text{dL}$, 2.66 ± 0.19) $\mu\text{g}/\text{dL}$ respectively. Increases from first to third trimester ranged from 1.6-5%. PbB concentrations significantly differed with location ($p=0.01$). Location mean ($\pm\text{SEM}$) was 2.27(± 0.13) $\mu\text{g}/\text{dL}$, 2.06(± 0.14) $\mu\text{g}/\text{dL}$, 2.18(± 0.30) $\mu\text{g}/\text{dL}$ and 3.60(± 0.48) $\mu\text{g}/\text{dL}$ in Serowe, Palapye, Maunatlala and Lerala respectively. The highest concentrations were observed in women from Lerala compared to Serowe ($p=0.02$) and Palapye ($p=0.01$). Mean ($\pm\text{SEM}$) values of women from Lerala were 3.33(± 0.86), 3.78(± 0.896) and 3.84(± 0.82) in the first, second and trimesters respectively. The increase in PbB levels was more a function of the socioeconomic status of women in Lerala village than a function of location. To the best of our knowledge this is the first study to measure PbB concentrations in pregnant women in Botswana. The significant increase in blood levels during the different stages of pregnancy in this study is of clinical importance and emphasizes the need for primary prevention interventions of exposure sources for lead during pregnancy to reduce lifetime lead exposure and decrease the risk for fetal lead exposure.

Key words: Blood-lead levels; pregnant women; Exposure; Central District; Botswana

5.2 Introduction

Lead is exclusively toxic to living organisms, including humans and as a result is associated with a range of adverse health effects to different segments of the population. Human exposure to lead can be from anthropogenic and natural sources. In addition life style, behavioural and occupational activities can be a contributing factor. Once in the blood stream, lead binds directly to erythrocytes, accumulates in the renal tubules, hepatocytes and eventually deposited in bone and teeth. It is estimated that 95% of the total lead burden in the body is stored in bone and that the half-life of lead in bone is 20-30 years while it is 1-2 months in blood¹⁴. Blood lead levels therefore only reflect the active, toxic fraction of lead and may be indicative of acute intoxication.¹⁵ Studies have estimated that mild mental retardation and cardiovascular outcomes resulting from exposure to lead amount to almost 1% of the global burden of disease, with the highest burden in developing regions.¹ Evidence also exists that lead contributes to mental retardation and neurotoxicity.^{2,3} Gastrointestinal effects and anemia are some of the earliest toxic effects of lead to be recognized; however, over the years it has become increasingly evident that the nervous system is the principal target for lead among others.⁴⁻⁷ Lower levels of lead decrease reaction time,^{8,9} cause deficits in hand-eye co-ordination,¹⁰ and decreased nerve conduction velocities.^{11,12} Severe lead poisoning results in adverse effects such as encephalopathy which can be followed by coma and death.¹³

Of major concern is lead exposure in pregnant women and risk of passing on the lead to the fetus. During pregnancy, a considerable blood exchange occurs between the mother and the fetus as the placenta does not provide sufficient protection from fetal exposure to lead and other toxic chemicals.¹⁶⁻¹⁸ Lead crosses the placenta through gestation and the correlation between maternal and umbilical cord -blood is estimated at 0.55 to 0.92.^{15,19} Studies have further found higher concentrations of lead in amniotic fluid than in cord blood suggesting that fetal membranes, which in these studies showed high concentrations of lead, may be absorbing lead from amniotic fluid.²⁰

5.3 Possible lead exposure sources in Botswana

Botswana, a mineral-rich country located in Southern Africa and a fast growing country is of particular importance to the assessment of highly ubiquitous heavy metals such as lead. Botswana only stopped the use of leaded petrol in 2005. In 1999, lead in fuel was estimated at 106 tons per year.²¹ Lead imported to Botswana is mainly in the form of lead oxides. It is also used as a stabilizer in PVC-manufacturing. The main distributors of paint in Botswana report that they do not use lead-based paint pigments currently. However, it is not clear when these distributors stopped using lead based paint pigments in particular because South Africa, (where most of the paints used in Botswana originate from), “white lead” in paint was abolished in the 1940s and a voluntary agreement reached with the paint industry to limit the use of leaded pigments in the 1970s. However, researchers in South Africa have detected lead in paint at levels above the set standards and report that legislation to regulate lead in paint has been introduced.²²⁻²⁴ Lead has also been detected in peeling paint in the city of Gaborone, Botswana.²⁵

Like many other developing nations, Botswana is burdened with infectious diseases such as TB, HIV/AIDS as well as malaria and other epidemics. These, combined with socioeconomic related issues, make populations in developing nations, more vulnerable to the toxic effects of pollutants in their living environments.²³ In 2009, the first ever study performed in Botswana on children lead exposure found that 31% of children in the city of Gaborone had blood lead levels $\geq 10\mu\text{g/dL}$.²⁵ The contribution of lead to the burden of disease in Botswana is currently unknown. Potential sources of lead exposure in the general population are also not known.

This study is part of a broader research assessing lead levels in pregnant women from the first trimester until six weeks after delivery. It aims to determine and measure the variation in blood lead levels at each stage of pregnancy in a randomly selected sample of pregnant women in four villages of different socioeconomic and environmental background. The study further establishes whether the levels of lead will be influenced by the type of location. A manuscript that will assess correlates of

blood lead levels from the first trimester until parturition is in preparation. The study area was selected deliberately because of its significance as a coal mining district.

5.4 Materials and methods

5.4.1 Study sites and participants

Women were recruited from Serowe, Palapye, Maunatlala and Lerala villages. Serowe is the largest of the villages with a population approximately 52,000,²⁶ a typical major village in Botswana with minimal industrial activity but moderate traffic volume. Palapye is a moderately to high industrial major village with a population of approximately 37,000,²⁶ located about 5-7 kilometers from Morupule coal mine and the Morupule Power Station, with a major railway station and a major highway passing through the village from Gaborone to Francistown (Figure 1). Maunatlala and Lerala with populations 4,552²⁶ and 687²⁶ respectively are classified as small rural villages in Botswana with no industrial activities and minimal traffic volume. Figure 5.1 shows the location of the study area.

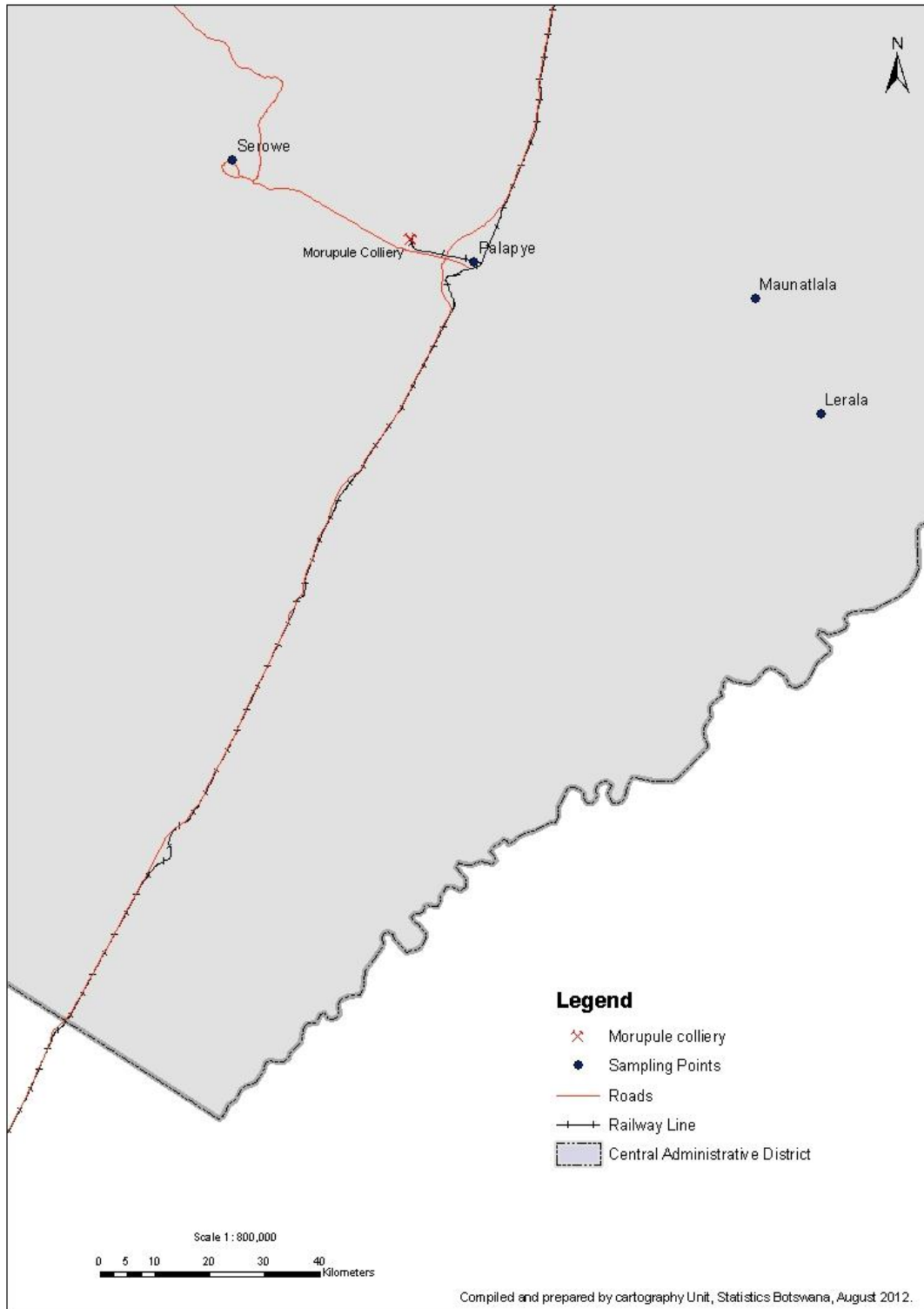


Figure 5.1: Central district sampling locations

5.4.2 Participation, recruitment and informed consent

All pregnant women registering for prenatal clinic between September 2009 and February 2010 who were between 0 and 12 weeks of pregnancy were invited to take part in the study through information leaflets developed specifically for the study and

through public addresses at the participating facilities whilst the women were waiting to be served. *A priori* exclusion criteria for the study were: 1) younger than 18 and not older than 49 years; 2) active diabetes 3) hypertension that was managed with medication and 4) any medical problem known to the health worker screening the pregnant woman that would compromise their health if they took part in the study. Women read, or were read the informed consent and agreed to participate.

The original population for the study comprised of 200 pregnant women who expressed interests to donate blood at each trimester of pregnancy and six weeks after delivery. Of these 200, 36 were not eligible and therefore excluded from the outset of the study. From the 164 eligible women, 28(17%) could not donate blood at the first trimester due to medical reasons (21%) or showing up at the health facility after 12 weeks had elapsed (60%) or discovered to be under-age upon age verification at the time of the first blood draw (18%). Overall a total of 137, 126 and 106 women enrolled and continued with the study on the first, second and third trimesters respectively as elaborated on Figure 2. Once having consented, outpatient staff screened the women to assess if their health will allow a blood draw. A midwife was employed to follow up the pregnant women and liaise with participating facilities. A qualified phlebotomist was also employed for the study to ensure the safety of the pregnant women and quality control issues in blood samples collected for the study.

5.4.3 Ethical Considerations

This study obtained unconditional ethical approval from the Research Ethics Committee, Faculty of Health Sciences, University of Pretoria, South Africa (reference 110/2009) and endorsement by the Ministry of Health Research and Ethics Committee, Gaborone, Botswana in 2009

5.4.4 Research Instrument

Validated risk assessment questionnaires were administered after the consent forms were signed by study participants. Participants were asked about their personal habits during the first trimester of pregnancy such as alcohol consumption, tobacco use and traditional medicine and whether they have pica behavior. In terms of practices, women were asked what substances they use to treat skin or other health related

problems during pregnancy. Follow up question on personal habits, diet or health status were asked when the women came for subsequent blood samples. To promote trust among the research participants and create a sense of ownership to the study, a feedback workshop was held with all study participants after the first draw of blood. The workshop was intended to inform the participating women on the progress of the study and emphasize the importance of the study on potential benefits not only to their health but the health of other women in reproductive age. This helped in maintaining the women in the study as can be observed in figure 5.2 that the loss to follow up rate was less than in any other trimester.

5.4.5 Sample collection and analysis

5.4.5.1 Maternal blood collection

Blood samples were drawn by venipuncture at intervals of 8-12 weeks, 20-24 weeks, and 34-36 weeks of pregnancy. The final sample was collected six weeks after delivery (data reported in Chapter 6). Each participant was given a unique identifying number in a sticker placed on their obstetric record booklet for easy identification by the outpatient staff when the patient comes for follow up. The same number was placed on the consent form for identification purpose of the results. The timing for the blood draw was synchronized with the schedule for follow up in the obstetrics record as well as the timing for risk assessment. Each pregnant woman donated 15 ml of blood consisting of 2 venous samples of 7.5 mL each (Venipuncture Needle-Pro, SIMS Portex, Inc, Keene, NH). Each venous blood sample was drawn into a Vacutainer tube (K₃ EDTA, BD 36-9651; Becton Dickinson, Rutherford, NJ) labeled with a code for identification. The sampling procedures followed the protocols prescribed by the National Institute of Occupational Health (NIOH), Johannesburg, South Africa where analysis was done.

5.4.5.2 Determination of lead in maternal blood

The analyses for lead content in whole blood were performed using graphite furnace spectrometry (Perkin-Elmer AAnalyst – 600) instrument. Contamination free vessels and certified reference standards were used throughout the analyses.

Briefly, blood samples diluted ten times with Triton X (0.1%), and mixed on a vibration mixer. The preparation of calibration standards included the addition of a

1ppm lead standard to ox blood and Triton-X to a final volume of 5 ml. The range of the calibration standards prepared was 5 – 80 µg/dl. The standards (10µl), samples (10 µl) and quality controls (10 µl) were analysed on a Perkin-Elmer AAnalyst – 600. The instrument parameters include: wavelength 283.3, the furnace temperature was set initially at 50°C and then ramped to 2500°C, run time: 2 min. Certified Reference Material, Nycomed Seronorm Trace Element control level 1 and 2 as well as in-house prepared reference controls were analyzed after every 10 samples. The detection limit for the blood lead analysis was 1 µg/dl. The coefficient of variation for Seronorm level 1 was 14.02 % and level 2 was 10.86%.

5.4.6 Statistical Analysis

Data was “double punched” using a Microsoft Excel software package, and subsequently transferred to STATA 11.0 statistical programme using Stattransfer. Statistical analysis was performed separately for each trimester and on the entire data set. This was to enable us to determine significant changes as well as trends in lead through pregnancy. This approach also allowed us to account for intrinsic variables associated with lead at each trimester of pregnancy. Descriptive statistic was calculated for lead which included the mean and Standard Error of Mean (SEM), the median and the range. We used Kruskal Wallis and Dunn tests for multiple comparisons. A significant difference was defined by a p value of <0.05.

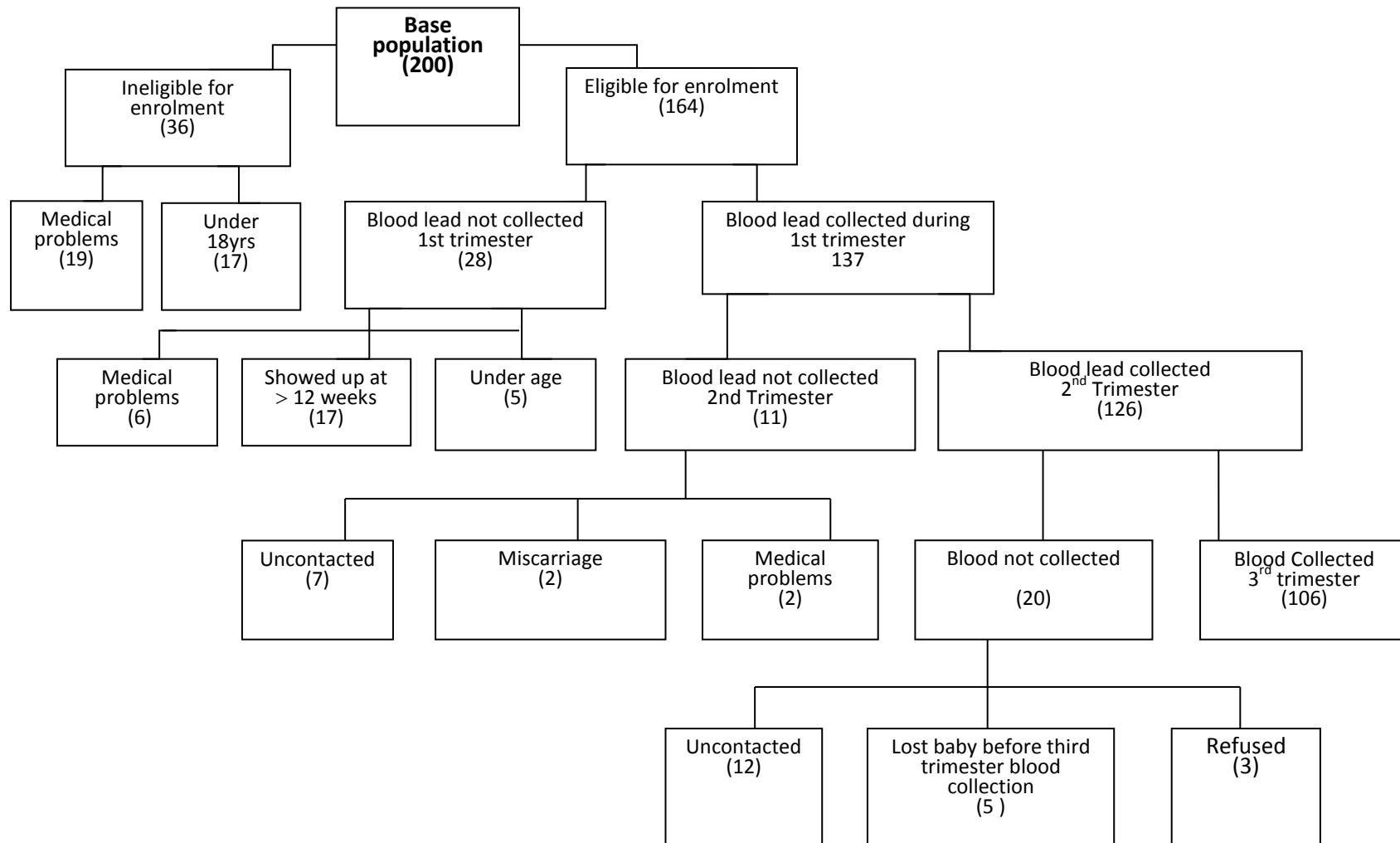


Figure 5.2 Schematic diagram of the study population and recruitment process

5.5 Results

5.5.1 Socioeconomic and demographic characteristics of participants

Table 5.1 summarizes socioeconomic and demographic characteristics of the women who continued with the study at each trimester (n= 137, 126 and 106 first, second and third trimesters respectively). The mean age of women was 27 years (range 18-44 years). Most of the study participants were young in their early to late 20s (approximately 57% across all trimesters). Approximately 34% of the women were 30 years and above and 9% aged below 20 years. Over 50% of the women were unemployed and relied on social grants or support from their partners or family members. The majority of women (62%) had 2-4 children.

Statistic	Trimester 1	Trimester 2	Trimester 3
Age (Years)	n=137	n=126	n=106
≤19	9.5	10.3	8.5
20-24	34.3	34.9	35.8
25-29	22.6	21.4	21.7
30-34	19.0	19.0	18.9
35 plus	14.6	14.3	15.1
Marital Status			
Single	88.3	89.7	88.7
Married	11.7	10.3	11.3
Educational status (in years)			
Primary Education (1-7 years)	13.1	13.5	15.1
Secondary Education (8-12)	64.2	65.1	64.2
Post-Secondary (13+years)	22.6	21.4	20.8
Employment			
Employed‡	43.1	42.1	42.5
Unemployed	56.2	57.1	56.6
Income adequacy‡			
Lowest (P 0.00-P3000)	93	93.7	92.5
Lower Middle(P3001-6000)	4.4	4.0	4.7
Middle (P6001-9000)	1	1	1
Upper Middle(9001-12000)	1	1	1
Parity			
Primipara	39.4	38.9	36.8
Multipara	60.6	61.1	63.2
Site			
Serowe (Major Village)	44.4	46.0	45.3
Palapye (Major village with industrial activity)	34.3	34.1	32.1
Maunatlala (Small rural Village)	8.8	9.5	10.4
Lerala (Small rural village)	9.5	10.3	12.3
Recruitment Facility type			
Hospital	27.7	26.2	27.4
Clinic	72.3	73.8	72.6

‡One participant did not indicate their employment status

It must be noted that the socioeconomic characteristics reported here differ with the one on chapter 3 as we wanted to establish the characteristics of women who donated blood from trimester 1 to 3.

Women from Lerala (a small village) had the most children (92%) compared to women from Maunatlala (a small village - 72%) and major villages (Serowe and Palapye - approximately 45%). Compared to major villages, women from small villages were significantly multiparous ($p=0.05$). Employment rate was significantly different between major villages and small villages ($p<0.001$) with Lerala having 80% of women employed compared to major villages. However, the employed women from Lerala earned between Botswana Pula 500 (USD71) and 1500 (USD214), which placed them in the lowest income adequacy grouping. Only one woman was employed from Maunatlala. Income adequacy was not significantly different between small and major villages even though major villages had higher absolute values compared to small villages.

5.5.2 Housing and living environment of participants

Table 5.2 summarizes housing and environmental characteristics of the women. The type of fuel used for cooking and lighting is significantly different among locations. Women in small rural villages predominantly use wood than women in major villages ($p=0.003$).

Statistic	Serowe (n=65)	Palapye (n=47)	Maunatlala (n=12)	Lerala (n=13)
Home Ownership	67	66	100	84
Heating**				
Electricity	12.3	8.5	-	7.7
Paraffin	4.6	-	-	-
Gas	18.5	38.3	-	1.0
Wood	27.7	17.0	66.6	76.9
Car battery	1.5	2.1	0	-
Gas/wood	18.5	27.7	25	-
Electricity/gas	16.9	6.4	8.3	7.7
Water Source				
Indoor tap	26.2	29.8	10	-
Outdoor tap	66.2	68.1	81.7	100
Rainwater	3.1	-	-	-
Outdoor/indoor tap	4.6	2.1	8.3	-
Type of plumbing				
Plastic	23.1	31.9	-	7.7
Metal	20	59.6	91.7	92.2

Palapye women used gas more and less of wood than all other areas depicting its semi urban nature. Home ownership was higher in small villages. Compared to all villages, women from Lerala relied solely on outdoor public taps for their water supply.

5.5.3 Behaviors and practices of participants

Table 5.3 summarizes behavioral characteristics of the women by site. The general characteristics of the women who participated in the study were proportional throughout the three trimesters..

Table 5.3 Behaviour and Practices of Participants			
Statistic	Trimester 1 (%)	Trimester2 (%)	Trimester 3 (%)
Alcohol Consumption			
Serowe (T1, N=65; T2,N=58; T3, N=48)	47.7	46.6	47.9
Palapye (T1, N=47; T2,N=43; T3, N=34)	40.8	39.5	44.1
Maunatlala (T1, N=12; T2,N=12; T3, N=11)	66.7	66.7	63.6
Lerala (N=13 all trimesters)	61.5	61.5	61.5
Tobacco Use¶			
Serowe (T1, N=65; T2,N=58; T3, N=48)	9.2	10.3	10.4
Palapye (T1, N=47; T2,N=43; T3, N=34)	4.3	4.7	5.9
Maunatlala (T1, N=12; T2,N=12; T3, N=11)	8.3	8.3	9.1
Lerala (N=13 all trimesters)	7.7	7.7	7.7
Geophagia			
Serowe (T1, N=65; T2,N=58; T3, N=48)	60	58.6	56.2
Palapye (T1, N=47; T2,N=43; T3, N=34)	46.8	44.2	47.1
Maunatlala (T1, N=12; T2,N=12; T3, N=11)	33	33	27.3
Lerala (N=13 all trimesters)	76.9	76.9	76.9
Paint Chip Pica			
Serowe (T1, N=65; T2,N=58; T3, N=48)	3.1	1.7	2.1
Palapye (T1, N=47; T2,N=43; T3, N=34)	6.4	7.0	8.8
Maunatlala (T1, N=12; T2,N=12; T3, N=11)	NR	NR	NR
Lerala (N=13 all trimesters)	NR	NR	NR
Matchstick sucking pica			
Serowe (T1, N=65; T2,N=58; T3, N=48)	15.4	15.5	14.6
Palapye (T1, N=47; T2,N=43; T3, N=34)	14.9	16.3	14.7
Lerala (N=13 all trimesters)	15.4	15.4	15.4
Pencil Pica			
Serowe (T1, N=65; T2,N=58; T3, N=48)	13.8	13.8	10.4
Palapye (T1, N=47; T2,N=43; T3, N=34)	8.5	9.3	8.8
Lerala (N=13 all trimesters)	7.7	7.7	7.7
Chalk Pica			



Serowe (T1, N=65; T2,N=58; T3, N=48)	1.5	1.7	2.1
Pica Bone Meal Pica			
Serowe (T1, N=65; T2,N=58; T3, N=48)	3.1	1.7	2.1
Brake Fluid Use for Psoriasis, ringworm treatment			
Serowe (T1, N=65; T2,N=58; T3, N=48)	40.0	37.9	35.4
Palapye (T1, N=47; T2,N=43; T3, N=34)	19.1	18.6	17.6
Maunatlala (T1, N=12; T2,N=12; T3, N=11)	33.3	33.3	36.4
Lerala (N=13 all trimesters)	30.8	30.8	30.8
Use of Traditional Cosmetic Clays (Letsoku) for skin Smoothing **			
Serowe (T1, N=65; T2,N=58; T3, N=48)	23.1	22.4	20.8
Palapye (T1, N=47; T2,N=43; T3, N=34)	10.6	9.3	11.8
Maunatlala (T1, N=12; T2,N=12; T3, N=11)	33.3	33.3	36.4
Use of light brown shoe-polish for skin smoothing and vanishing*			
Serowe (T1, N=65; T2,N=58; T3, N=48)	26.2	29.3	31.2
Palapye (T1, N=47; T2,N=43; T3, N=34)	6.4	7.0	8.8
Maunatlala (T1, N=12; T2,N=12; T3, N=11)	16.7	16.7	18.2
Lerala (N=13 all trimesters)	23.1	23.1	23.1
Use of Torch Battery contents to treat ringworm			
Serowe (T1, N=65; T2,N=58; T3, N=48)	12.3	13.8	16.7
Palapye (T1, N=47; T2,N=43; T3, N=34)	2.1	2.3	2.9
Maunatlala (T1, N=12; T2,N=12; T3, N=11)	8.3	8.3	9.1
Lerala (N=13 all trimesters)	7.7	7.7	7.1
Use of Traditional Herbs			
Serowe (T1, N=65; T2,N=58; T3, N=48)	18.8	15.5	16.9
Palapye (T1, N=47; T2,N=43; T3, N=34)	5.9	7.0	6.4
Maunatlala (T1, N=12; T2,N=12; T3, N=11)	9.1	8.3	8.3
Lerala (N=13 all trimesters)	7.7	7.7	7.7
Use of over the Counter Drugs			
Serowe (T1, N=65; T2,N=58; T3, N=48)	27.1	22.4	21.5
Palapye (T1, N=47; T2,N=43; T3, N=34)	23.5	27.9	27.7
Maunatlala (T1, N=12; T2,N=12; T3, N=11)	9.1	8.3	8.3
Fasting During Pregnancy***			
Serowe (T1, N=65; T2,N=58; T3, N=48)	22.9	25.9	24.6
Palapye (T1, N=47; T2,N=43; T3, N=34)	2.1	4.7	4.3
Maunatlala (T1, N=12; T2,N=12; T3, N=11)	9.1	8.3	8.3
Lerala (N=13 all trimesters)	NR	NR	NR
T1=Trimester 1;T2=Trimester 2;T3=Trimester 3			
¶Only one woman from Palapye reported smoking. The rest were using snuff			
***Significant difference observed in all trimesters (p<0.05)			
** Significant difference observed in trimesters 1 and 2 only (p<0.05)			
*Significant difference observed in the second trimester only (p<0.05)			
**Significantly different (p=0.003)			

There were generally no significant differences in the behaviors of women by site with the exception of the use of *letsoku*, a traditional clay cosmetic, the use of shoe polish for skin smoothing, and fasting during pregnancy ($p < 0.05$). In all cases women from Serowe were more likely than other women to engage the use of *letsoku*, shoe polish as a beautifying product and fasting during pregnancy. However, alcohol consumption was more prevalent in women from small rural villages compared to major villages.

Geophagia (the intentional ingestion of soils) was the most reported practice across all locations, but highest in Lerala village (approximately 77%) even though the difference was not significant ($p > 0.05$). Chalk, bone meal and paint chips pica were the least practiced habits, and mainly reported in Serowe. The ingestion of paint chips by pregnant women was only prevalent in major villages. Brake fluid, reported to be used for treatment of psoriasis and ringworm was evidently less prevalent in Palapye

5.5.4 *Self-reported dietary intake of selected food items*

Table 5.4 summarizes self-reported dietary intake frequency of selected iron, calcium and protein rich foods. Women in smaller villages consumed wholegrain foods more frequently than those in urban areas ($p < 0.001$), while consumption of green leaf vegetable and meat cut across all sites except in Lerala where meat was consumed less. Generally, Lerala women performed poorly in terms of dietary requirements compared to all other villages. Maunatlala women generally reported a sufficient dietary intake compared to Lerala and the two major villages. The consumption of calcium rich food such as milk was highest in Maunatlala followed by Palapye on average. Calcium and iron supplement consumption was not reported by women in Lerala whilst it was highest in Palapye and Serowe (average 50%) and Maunatlala (average 40%).

Table 5.4: Self-reported information on dietary intake selected food items (%)

Statistic	Serowe (n=65)	Palapye (n=47)	Maunatlala (n=12)	Lerala (n=13)
Wholegrain**				
Daily	7.7	17	50	30.8
2-3X a week	30.8	17	25	0
1-4X a month	33.8	48.9	16.7	15.4
Green leaf vegetables				
Daily	50.8	29.8	58.3	38.5
2-3X a week	30.8	53.2	41.7	38.5
1-4X a month	13.8	10.6	0	23.1
Red Meat				
Daily	43.1	31.9	45.5	0
2-3X a week	21.5	38.3	27.3	60
1-4X a month	16.9	19.1	9.1	10
Milk				
Daily	26.2	34	41.7	15
2-3X a week	21.5	21.3	41.7	30.8
1-4X a month	21.5	25.5	8.3	0
Ice cream**				
Daily	9.4	10.6	8.3	7.7
2-3X a week	32.8	23.4	0	0
1-4X a month	32.8	53.2	75.0	15.4
Fish				
Daily	0	4.3	8.3	0
2-3X a week	30.8	36.2	50.0	0
1-4X a month	35.9	34.0	25.0	38.5
Yoghurt				
Daily	9.2	8.5	8.3	0
2-3X a week	30.8	29.8	25	1
1-4X a month	33.8	44.7	50	23.1
Dietary Supplements**	46.2	21.3	83.3	61.5
Type of Supplement taken**				
Iron	87.5	50	60	0
Calcium	12.5	50	20	0
Multivitamins	0	0	20	100

** Significantly different (p<0.05)

5.5.5 Blood lead levels of pregnant women across trimesters

Table 5.5 summarizes concentrations of lead in whole blood by site and trimester. PbB concentrations ranged from 0.5 to 12.90µg/dL with an overall mean (\pm SEM) of 2.34 (\pm 0.098) µg/dL. PbB concentrations of \geq 5µg/dL were observed in 5.5%, 5.6% and 3.1% of women during the first, second and third trimesters of pregnancy. PbB concentrations significantly differed among locations (p=0.01) (Figure 3). Overall mean (\pm SEM) were 2.27(\pm 0.13) µg/dL, 2.06 (\pm 0.14) µg/dL, 2.18(\pm 0.30) µg/dL and 3.60(\pm 0.48) µg/dL in Serowe, Palapye, Maunatlala and Lerala respectively. The highest concentrations were observed in women from Lerala compared to Serowe (p=0.02) and Palapye (p=0.01). No significant differences were observed between Lerala and Maunatlala. Mean (\pm SEM)

Table 5.5 Blood lead levels by site and trimester ($\mu\text{g}/\text{dL}$)				
Statistic	Serowe (T1, N=65; T2,N=58; T3, N=48)	Palapye (T1, N=47; T2,N=43; T3, n=34)	Maunatlala (T1, N=12; T2,N=12; T3, N=11)	Lerala (N=13 all trimesters)
Trimester 1				
Mean (SEM)	1.88(0.18)	1.75(0.19)	1.77(0.49)	3.33(0.86)
95% CI	1.53:2.23	1.38:2.13	0.69:2.84	1.46:5.20
Median	1.40	1.50	1.56	1.90
Range	0.50-5.40	0.50-5.60	0.50-6.80	0.50-11.80
Trimester 2				
Mean (SEM)	2.52(0.25)	2.16(0.26)	2.09(0.355)	3.78(0.896)
95% CI	2.03:3.01	1.64:2.68	1.31:2.87	1.83:5.74
Median	1.60	1.80	2.05	3.46
Range	0.50-7.50	0.50-8.40	0.50-4.40	0.50-12.90
Trimester 3				
Mean (SEM)	2.51(0.23)	2.38(0.32)	2.73(0.69)	3.84(0.82)
95% CI	2.05:2.91	1.74:3.02	1.20:4.27	2.04:5.62
Median	2.20	2.15	1.70	2.40
Range	0.50-8.50	0.50-10.30	0.50-6.80	1.00-9.80
SEM=Standard Error of Mean 95%CI =95% Confidence Interval T1=Trimester 1;T2=Trimester 2; T3=Trimester 3				

values of women from Lerala were $3.33(\pm 0.86)$, $3.78(\pm 0.896)$ and $3.84(\pm 0.82)$ in the first, second and trimesters respectively. The range was 0.50- 12.90 $\mu\text{g}/\text{dL}$.

Blood lead levels increased by trimester. A significant increase in PbB levels was observed between the first and third trimester ($p=0.01$) (Figure 4). However, no significant difference was observed between the first and second trimester ($p=0.07$). The mean PbB ($\pm\text{SEM}$) for the first, second and third trimesters were $1.96(\pm 0.14)$ $\mu\text{g}/\text{dL}$, $2.49(\pm 0.17)$ $\mu\text{g}/\text{dL}$, $2.66(\pm 0.19)$ $\mu\text{g}/\text{dL}$ respectively. The proportion of PbB between the first second and third trimester was 1.6%, 3.3%, 3.6% and 5.4% in Lerala, Serowe, Palapye and Maunatlala.

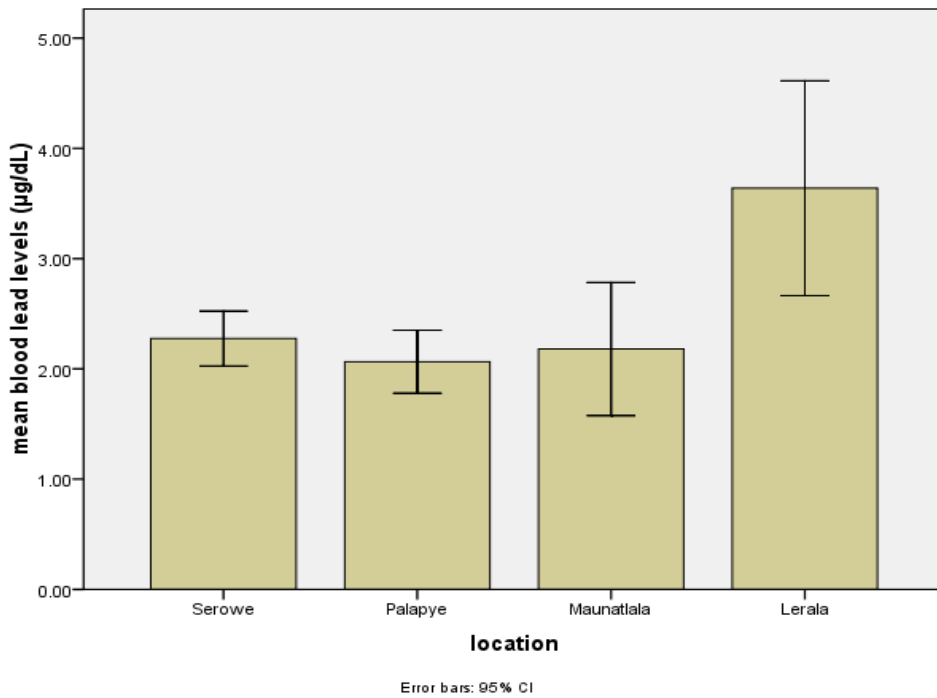


Figure 5.3 :Mean blood lead levels by location

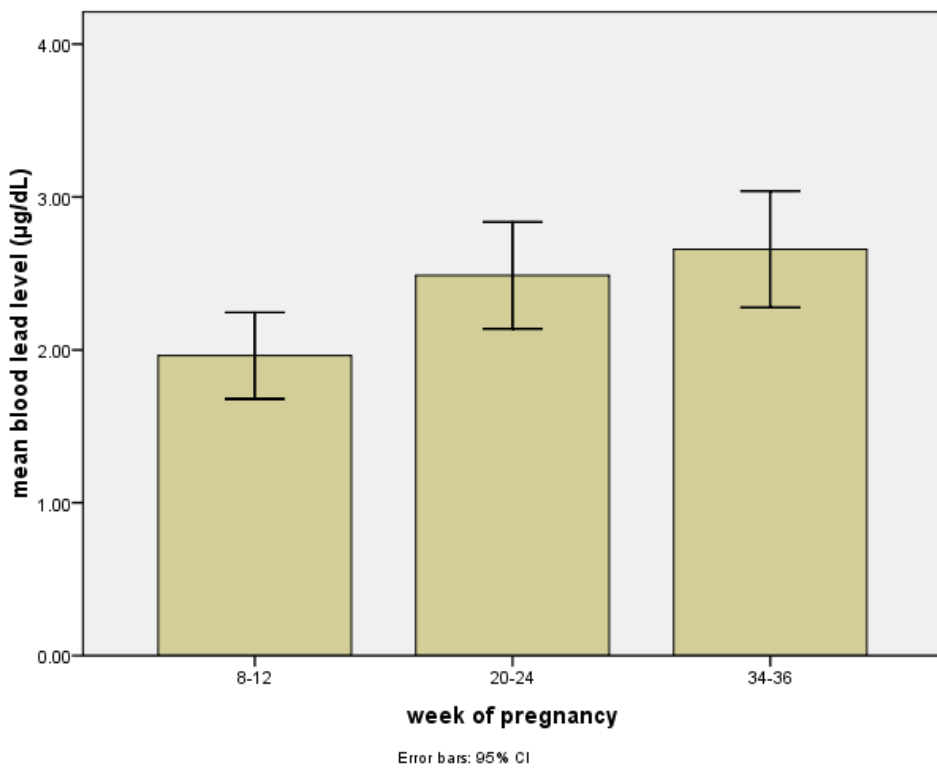


Figure 5. 4: Mean blood lead levels by week of pregnancy

5.6 Discussion

In this study we examined PbB concentrations among women aged 18 to 44 years to establish if women from areas of different socioeconomic and environmental background will have significant differences in PbB and to investigate if blood levels will differ by trimester in the Central Administrative District of Botswana. We found that there is a significant difference in blood lead concentrations between small and major villages. Interestingly, we have found that women from a poor small rural village in Botswana had significantly higher PbB levels compared to women from major villages where moderate to high industrial activity takes place. Overall we also found that women in their third trimester had significantly higher blood PbB compared to their first trimester.

The highest mean PbB concentrations among the study sites were recorded at Lerala followed by Maunatlala during the third trimester. These findings could be explained by several factors. These two villages are both categorized as small and rural villages with the lowest income adequacy. For example, unemployment was highest in Maunatlala, and, even though the women in Lerala reported being employed their income earnings placed them in the lowest income adequacy category. Women from the two small villages also have the highest number of children compared to women from major villages. Additionally, women from Lerala village generally had a low dietary intake of calcium and iron rich foods. Iron and calcium supplementation was also very poor in Lerala compared to Maunatlala and other villages. This is despite the fact that every pregnant woman attending antenatal clinics in Botswana are automatically supplied with iron, ferrous sulphate, calcium and vitamin C. Lerala women were further reliant on outdoor public standpipe a compound standpipes supplied from boreholes by the water authorities. This water is purified however, our suspicion is that lead leaches are higher due to the high water temperature as a result of exposure to high heat from the outdoor environment. This could also be reflection of women not affording a water connection fee to municipal water supply or not affording to install water fittings in their houses due to poverty. Geophagia, the intentional ingestion of soils, was highest in Lerala and

alcohol consumption highest in both Maunatlala and Lerala. Based on these factors, it can therefore be concluded that the higher PbB concentrations in small villages are a function of environmental, socioeconomic and lifestyle behaviors.

Although this is the first study investigating lead exposure among pregnant women in Botswana, a study carried out in 2010 found 31% of children aged 1-6 years having PbB levels equal to or exceeding the action level of 10 $\mu\text{g/dL}$. The main confounding factor was maternal unemployment.²⁵ Our results are consistent with the said study and studies conducted elsewhere which have found strong relationships between populations of poorer socioeconomic status and exposure to lead.²⁷⁻²⁹

Diet is an important component in the absorption of lead. Deficiencies of calcium and iron have been found to enhance the absorption of lead.^{30,31} Evidently, women from Lerala had low dietary calcium and iron. As a result we suspect that the low calcium diet and not taking calcium supplements has contributed to the enhancement of gastrointestinal lead absorption. This is consistent with other studies.³¹ It is worth noting that whilst the government provides supplements to pregnant women, we observed that many of the women collected the supplements but did not use them. Further probing of the women revealed the women said the supplements make them sick. This finding reveals the need for awareness and education of pregnant women on the importance of the supplements provided.

Women in small villages, Lerala and Maunatlala, are geophagic as well as consuming alcohol more than women from major villages. Studies have proven that infants born to women who smoke, drink and maintain a poor nutritional status for selected nutrients are at a greater risk of lead toxicity than those born to other women.³² Severe lead poisoning has also been observed in women who are geophagic.^{33,34}

Studies have reported a significant increase in maternal PbB during periods corresponding to 20-36 weeks.^{35,36} This study has reflected a similar trend with a significant increase in blood lead levels between the first and the third trimester. An increase of PbB concentrations in the last half of pregnancy is reported to coincide with an increased fetal need for calcium as well as an increased maternal provision of calcium.³⁷ The resultant effect is that if the needed supply of calcium is supplied from the expectant woman's bone, then women with high loads of bone lead may transfer more lead to the bloodstream with calcium. In this study PbB concentrations increases from the first to the third trimester ranged from 1.6 to 5%. The lowest increase between trimester 1 and trimester 2 was observed in women from Lerela who had the highest number of children prior to the current pregnancy. There is evidence that if the fetus transports maternal lead from the mother's body (bone) stores, blood lead levels will be lowered in subsequent pregnancies.³⁵ A similar trend in is observed in this study using the example of Lerela women who starting off with significantly higher levels, but resulting with a small 1.6% increase in PbB on the third trimester compared to Maunatlala for example that started off with lower blood lead levels in the first trimester but ending with a 5% increase on the third trimester. These findings are similar to other studies that have found multigravida women to have lower PbB levels during the last trimester (relative to lead levels in the first trimester).³⁵

5.7 Limitations:

As with any progressive cohort studies women were lost in each trimester of pregnancy. Additionally as in the previous chapters, the lack of resources could not allow sampling in an urban area. This could have provided a good comparison of trends among women in villages and urban areas.

The study did not assess knowledge and attitudes on lead exposure or lead exposure sources. This could have provided an insight as to whether women engage in the practices and behaviours knowingly. Future studies should assess such.

5.8 Conclusions

To the best of the author's knowledge, this is the first ever study conducted in Botswana that measured blood lead concentrations in pregnant women at each stage of their pregnancy. The results suggest that location and socioeconomic factors have an influence on PbB concentrations during pregnancy. Even though we did not investigate knowledge and health beliefs, the results further suggest that most women are ignorant of lead and its potential negative impacts on pregnancy outcomes. This was confirmed during workshops with study participants as well as during interactions with them through interviews. Most importantly, the results suggest that women who have been exposed to lead continuously may have higher PbB circulating in their blood.

Currently the Government of Botswana does not have in place any system to keep track of lead exposure. Such systems will be necessary to assess risks to health and to determine trends in exposure to lead over time. Public education and awareness is crucial in the prevention of lead exposure. It is important therefore for the Government to develop interventions that would reach most vulnerable population groups such as young children, young adults and women in reproductive age to prevent lead exposure in the early years of their lives and thus prevent fetal lead exposure.

Training and education of health care workers, parents and the communities at large should be intensified and food supplementation programmes should be promoted. Pregnant women should be made aware of lead and the importance of good nutrition practices, avoid potential lead containing substances to avert serious medical consequences to their health and that of their unborn children

5.9 References

1. Fewtrell L, Kaufmann R, Prüss-Üstün A. Lead: Assessing the environmental burden of disease at national and local levels. 2003.
2. Tong S, McMichael AJ. The magnitude, persistence and public health significance of cognitive effects of environmental lead exposure in childhood. *J Env Med* 1999;1:103-110.

3. Rothenberg SJ, Schnaas L, Cansino-Ortiz S, Perroni-Hernandez E, de la Torre P, Neri-Mendez C, et al. Neurobehavioral deficits after low level lead exposure in neonates: the Mexico City pilot study. *Neurotoxicol.Teratol.* 1989 Mar-Apr;11(2):85-93.
4. Bellinger DC. Neurological and behavioral consequences of childhood lead exposure. *PLoS Med.* 2008 May 27;5(5):e115.
5. Goyer RA. Results of lead research: prenatal exposure and neurological consequences. *Environ.Health Perspect.* 1996 Oct;104(10):1050-1054.
6. Todd AC, Wetmur JG, Moline JM, Godbold JH, Levin SM, Landrigan PJ. Unraveling the chronic toxicity of lead: an essential priority for environmental health. *Environ.Health Perspect.* 1996 Mar;104 Suppl 1:141-146.
7. Goyer RA. Lead toxicity: current concerns. *Environ.Health Perspect.* 1993 Apr;100:177-187.
8. Needleman H. Lead Poisoning. *Annu.Rev.Med.* 2004 02/01;55(1):209-222.
9. Chiodo LM, Covington C, Sokol RJ, Hannigan JH, Jannise J, Ager J, et al. Blood lead levels and specific attention effects in young children. *Neurotoxicol.Teratol.* 2007 Sep-Oct;29(5):538-546.
10. Stokes L, Letz R, Gerr F, Kolczak M, McNeill FE, Chettle DR, et al. Neurotoxicity in young adults 20 years after childhood exposure to lead: the Bunker Hill experience. *Occup.Environ.Med.* 1998 Aug;55(8):507-516.
11. Needleman HL, Bellinger D. The health effects of low level exposure to lead. *Annu.Rev.Public Health* 1991;12:111-140.
12. Landrigan PJ, Baker EL,Jr, Feldman RG, Cox DH, Eden KV, Orenstein WA, et al. Increased lead absorption with anemia and slowed nerve conduction in children near a lead smelter. *J.Pediatr.* 1976 Dec;89(6):904-910.
13. Goyer RA. Lead toxicity: from overt to subclinical to subtle health effects. *Environ.Health Perspect.* 1990 Jun;86:177-181.
14. Rabinowitz MB. Toxicokinetics of bone lead. *Environ.Health Perspect.* 1991 Feb;91:33-37.
15. Wong GP, Ng TL, Martin TR, Farquharson DF. Effects of low-level lead exposure in utero. *Obstet.Gynecol.Surv.* 1992 May;47(5):285-289.
16. Goyer RA. Transplacental transport of lead. *Environ.Health Perspect.* 1990 Nov;89:101-105.
17. Ong CN, Phoon WO, Law HY, Tye CY, Lim HH. Concentrations of lead in maternal blood, cord blood, and breast milk. *Arch.Dis.Child.* 1985 Aug;60(8):756-759.
18. Wan BJ, Zhang Y, Tian CY, Cai Y, Jiang HB. Blood lead dynamics of lead-exposed pregnant women and its effects on fetus development. *Biomed.Environ.Sci.* 1996 Mar;9(1):41-45.

19. Ernhart CB. A critical review of low-level prenatal lead exposure in the human: 1. Effects on the fetus and newborn. *Reprod.Toxicol.* 1992;6(1):9-19.
20. Korpela H, Loueniva R, Yrjanheikki E, Kauppila A. Lead and cadmium concentrations in maternal and umbilical cord blood, amniotic fluid, placenta, and amniotic membranes. *Am.J.Obstet.Gynecol.* 1986 Nov;155(5):1086-1089.
21. Ministry of Health, Environmental Health Unit . Support to management of chemicals: Assessment of risks to related to use of selected prio ritized chemical substances in Botswana. 1999.
22. Mathee A, Rollin H, Levin J, Naik I. Lead in paint: three decades later and still a hazard for African children? *Environ.Health Perspect.* 2007 Mar;115(3):321-322.
23. Rollin HB, Rudge CV, Thomassen Y, Mathee A, Odland JO. Levels of toxic and essential metals in maternal and umbilical cord blood from selected areas of South Africa--results of a pilot study. *J.Environ.Monit.* 2009 Mar;11(3):618-627.
24. Montgomery M, Mathee A. A preliminary study of residential paint lead concentrations in Johannesburg. *Environmental Research*, 2005 7;98(3):279-283.
25. Mbongwe B, Barnes B, Tshabang J, Zhai M, Rajoram S, Mpuchane S, et al. Exposure to lead among children aged 1-6 years in the City of Gaborone, Botswana. *J.Environ.Health Res.* 2010;10(1):17-26.
26. Central Statistics Office Botswana. 2011 Botswana Population and Housing Census, Alphabetical Index of Districts . *Stats Brief* 2011 February 2007;01/2007:A1-12.
27. Mahaffey KR, Annest JL, Roberts J, Murphy RS. National estimates of blood lead levels: United States, 1976-1980: association with selected demographic and socioeconomic factors. *N.Engl.J.Med.* 1982 Sep 2;307(10):573-579.
28. Dietrich KN, Krafft KM, Bornschein RL, Hammond PB, Berger O, Succop PA, et al. Low-level fetal lead exposure effect on neurobehavioral development in early infancy. *Pediatrics* 1987 Nov;80(5):721-730.
29. Bellinger D, Leviton A, Waternaux C, Needleman H, Rabinowitz M. Low-level lead exposure, social class, and infant development. *Neurotoxicol.Teratol.* 1988 Nov-Dec;10(6):497-503.
30. Blake KC, Mann M. Effect of calcium and phosphorus on the gastrointestinal absorption of 203Pb in man. *Environ.Res.* 1983 Feb;30(1):188-194.
31. Heard MJ, Chamberlain AC. Effect of minerals and food on uptake of lead from the gastrointestinal tract in humans. *Hum.Toxicol.* 1982 Oct;1(4):411-415.
32. Lee MG, Chun OK, Song WO. Determinants of the blood lead level of US women of reproductive age. *J.Am.Coll.Nutr.* 2005 Feb;24(1):1-9.
33. Shannon M. Severe lead poisoning in pregnancy. *Ambul.Pediatr.* 2003 Jan-Feb;3(1):37-39.

34. Klitzman S, Sharma A, Nicaj L, Vitkevich R, Leighton J. Lead poisoning among pregnant women in New York City: risk factors and screening practices. *J.Urban Health* 2002 Jun;79(2):225-237.
35. Rothenberg SJ, Karchmer S, Schnaas L, Perroni E, Zea F, Fernandez Alba J. Changes in serial blood lead levels during pregnancy. *Environ.Health Perspect.* 1994 Oct;102(10):876-880.
36. Moura M, Goncalves Valente J. Blood lead levels during pregnancy in women living in Rio de Janeiro, Brazil. *Sci.Total Environ.* 2002 Nov 1;299(1-3):123-129.
37. Kumar R, Cohen WR, Epstein FH. Vitamin D and calcium hormones in pregnancy. *N.Engl.J.Med.* 1980 May 15;302(20):1143-1145.