

# Trace Element Concentrations in the Sera of Pregnant Women in Kisangani, Democratic Republic of Congo

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## Abstract

**Introduction:** During pregnancy, the increased demand for all the nutrients required for the development of the foetus means that the maternal stock of trace elements and minerals may become unbalanced if dietary intake fails to compensate. The aim of this research is to determine the status of trace elements (calcium, copper, magnesium, selenium and zinc) in pregnant women in the city of Kisangani. **Methods:** We carried out an analytical cross-sectional study of non-pregnant and pregnant women living in Kisangani, from 05 October 2023 to 05 January 2024. Concentrations of trace elements in sera were analysed using an inductively coupled plasma mass spectrophotometer (ICP-MS Agilent 7700X). Statistical analysis of the data was performed using R software version 4.3.0. **Results:** The mean age was  $26.3 \pm 6.7$  years; the mean copper and magnesium levels in the serum of pregnant women were  $12.58 \pm 1.13$  micromol/l and  $1.03 \pm 1.03$  mmol/l respectively. The medians for calcium and zinc were 1.49 mmol/l and 8.42 micromol/l. The selenium mode was 0.41 micromol/l. Variations in trace element levels in sera of pregnant women were 0.94 - 2.22 mmol/l for calcium; 0.31 - 0.78 micromol/l for selenium; and 7.29 - 12.72 micromol/l for zinc; 11.04 - 14.99 micromol/l for copper, and 0.082 - 1.05 mmol/l for magnesium. **Conclusion:** Serum trace element concentrations in pregnant women were lower than those observed in non-pregnant women. Trace element reserves in pregnant women depended on their nutrient status prior to pregnancy, hence, there was an urgent need for trace element balance prior to pregnancy.

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## Keywords

Trace Element, Zinc, Copper, Selenium, Calcium, Magnesium, Pregnant Woman, Serum, Kisangani

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## 1. Introduction

Trace elements are required in small quantities to support virtually all metabolic activities, including cell signalling, motility, proliferation, differentiation and apoptosis, which regulate tissue growth, function and homeostasis. Early in life, these fundamental biological roles enable the foetus to develop into a healthy newborn. Vitamins and trace elements support each stage of maternal, placental and foetal interaction to ensure a successful pregnancy [1].

Variations in trace element levels occur regularly in humans. The bioavailability, geographical location, soil content and dietary intake of trace elements significantly affect their status [2].

Maternal undernutrition, maternal mortality rates and infant mortality and morbidity rates have fallen since the 1990s due to the increasing focus on improving the quality of the antenatal period, better obstetric care and social change. However, further improvements are still greatly needed. A pregnant woman's nutritional status and size are the result of her past or pre-pregnancy health and nutrition [3].

Information on vitamin and mineral metabolism and requirements during pregnancy is surprisingly imprecise, largely due to the complexity of maternal metabolism during pregnancy, and interactions between trace elements [3] [4].

During pregnancy, the increased demand for all nutrients for the development of the foetus means that the maternal stock of trace elements and minerals would be out of balance if dietary intake failed to compensate [5]. Fetal mineral reserves are built up towards the end of pregnancy, although maternal trace element status can play an important role in the development of the mother-foetus pair from the start of pregnancy. Despite extensive research, the importance of trace element balance is still underestimated by many researchers.

For many years, in-depth research into micronutrient deficiencies in pregnant women has focused on common micronutrients such as folate and vitamin D [6]. However, there is now some evidence of the importance of trace elements such as iron, zinc, calcium, selenium and copper for a successful pregnancy [1].

Pregnancy is a period of increased metabolic demands, mainly due to changes in the woman's physiology and the demands of the growing foetus [7]. Oxygen consumption, central haemodynamic changes and oxidative stress are all altered, helping to determine the long-term health of the pregnant woman. Certain concentrations of trace elements are also altered during this period.

It should be noted that deficiency or overexposure to certain trace elements could be detrimental to the health of pregnant women and their foetuses [8].

It is important to understand how micronutrient status during the first trimester is associated with pregnancy outcome conditions (favourable or unfavourable), which are strongly influenced by the state of the maternal micronutrient reserve prior to pregnancy. Marginal micronutrient deficiencies early in pregnancy can lead to more severe deficiencies later in pregnancy due to the increased metabolic demands of the placenta and the rapidly growing foetus [9]. Much of the research into trace elements in pregnancy has been conducted at the end of pregnancy or at term. This means that the precursors of final deficiency, which can sometimes be life-threatening for the mother and foetus, go unnoticed [10].

The status of trace elements differs and/or varies between countries and regions of the world, and their bioavailability is strongly linked to their levels of absorption, dietary habits, lifestyles and environmental conditions. For this reason, the World Health Organisation would like each country or region of the world to establish or provide data on the status of essential trace elements and nutrients in relation to their environment and population, especially vulnerable populations such as pregnant and breastfeeding women, children and women of childbearing age [11].

The mother's nutritional status directly affects the growth and development of the foetus. Serum trace element levels are an important indicator of the mother's nutritional status during pregnancy, especially during antenatal visits, particularly in environments at risk of nutritional deficiency [12]-[14].

Data on trace element status in pregnant women is scarce in the Democratic Republic of Congo, and in Kisangani in particular. The medicines market in our area is full of molecules made up of multiple trace elements, and supplements are often given randomly without any knowledge of the possible deficiencies in pregnant women. That's why we initiated this research, with the aim of determining the current state of trace elements in pregnant women in Kisangani.

We asked ourselves what the values of trace elements are among pregnant women in Kisangani?

The aim of this research is to determine the status of trace elements (calcium, copper, magnesium, selenium and zinc) in pregnant women in the city of Kisangani, in order to improve their care in the event of a deficiency.

## **2. Methods**

### **2.1. Environment, Population and Type of Study**

We carried out an analytical cross-sectional study with prospective data collection at all the sites selected for this research.

This research took place in the town of Kisangani, in the north-east of the Democratic Republic of Congo, capital of the Tshopo province. Kisangani was chosen because of its technical facilities for assaying the trace elements used in this research, and the presence of a decentralized HUB KISANGANI depot with a hyper-cold chain (from  $-50^{\circ}\text{C}$  to  $-120^{\circ}\text{C}$ ) capable of storing sera in a stable state at  $-80^{\circ}\text{C}$ .

This was a multi-site study, carried out in 8 health facilities in Kisangani. These

were the Kisangani University Clinics, the Kabondo General Reference Hospital, the Reference Health Center of Matete, the Reference Health Center of Foyer, the Saint Joseph Health Center, the Mangobo General Reference Hospital, the Lubanga General Reference Hospital and the Makiso-Kisangani General Reference hospital. All these health facilities have outpatient, antenatal and maternity clinics, and medical biologists available to take venous blood samples. The particularity of these different sites is that they have a high frequency of sick people and pregnant women. And these health structures cover vast health areas with a large number of populations, and have a high attendance.

## 2.2. Population and Study Period

The study population consisted of non-pregnant and pregnant women living in the city of Kisangani. The study took place from 05 October 2023 to 05 January 2024.

## 2.3. Sample and Sampling

To calculate this sample size, we used the article “First trimester microelement and their relationships with pregnancy outcomes and complications” in Poland in 2020 published by Lewandowska M. *et al.* [15]. We used G-Power software version 3.1.9.7. with an  $\alpha$  threshold of 0.05 and 95% power, an effect size of 0.1. The minimum sample size was 1180 individuals, including 590 non-pregnant women and 590 pregnant women.

Our sampling was not exhaustive.

## 2.4. Selection Criteria

### 1) Inclusion criteria

- Be a woman of reproductive age living in the city of Kisangani;
- Pregnant women living in the city of Kisangani;
- Apparent good health;
- Have voluntarily agreed to take part in the study by signing the informed consent form;
- Have agreed to the blood sample being taken and personal data being recorded (Noted).

### 2) Non-inclusion criteria

- Breastfeeding or post-menopausal women living in the city of Kisangani;
- Women of childbearing age, inpatient or outpatient;
- Pregnant women with inpatient or outpatient morbidity;
- Non-pregnant or pregnant women who do not live in Kisangani.

### 3) Exclusion criteria

Non-pregnant woman or pregnant woman whose personal details have been incorrectly recorded, or the blood sample has been haemolyzed, or the sample (serum) has not been frozen at  $-80^{\circ}\text{C}$  within 3 hours of collection.

-If the number of blood samples taken was insufficient for all the laboratory tests.

## 2.5. Data Collection Process

Data collection was prospective in the outpatient departments of the selected hospitals. After a 48-hour training session with the research team (doctors, outpatient department head nurses, antenatal and maternity department heads, laboratory technicians and community relays).

The role of the community outreach workers was to mobilise non-pregnant women and pregnant women in the neighbourhoods and avenues to come to the hospital for screening; the doctors and nurses helped us to receive the women, explain the benefits of this activity, record their personal details and get them to sign the informed consent forms; the nurses took the vital signs and parameters. The laboratory assistants drew blood from the consenting women. The activities (from reception to sampling) took place every working day from 8.00 am to 1.00 pm (7.30 am to 2.30 pm on prenatal consultation days), depending on when the women arrived. Once the respondent had finished with the doctor and nurse, she was received for the sampling. The blood sample was taken in compliance with asepsis and antisepsis standards. The sampling site was disinfected with cotton-wool soaked in denatured alcohol. The sampling agent wore sterile gloves from the collection until the sample was placed in the clean isothermal box. Using a 5 ml syringe, a venipuncture was made in the forearm or the back of the hand, where 4-5ml of blood was taken. From the syringe, the blood was injected into a dry vacutainer tube, which was placed vertically in an isothermal box with an internal temperature of 2°C - 8°C. The principal investigator came round between 10:30 am and 2:30 p.m. to assess the day's work, check the status of the blood samples and fill in the forms.

At the end of the sampling, the samples were sent to the laboratory of the Kisangani university clinics. The tubes containing the blood were centrifuged at 5000 rpm for 5 minutes. The sera were then collected and divided by 2, placed in 2 different cryotubes and marked (each cryotube had a code identifying the woman in accordance with the identification form, *i.e.* each woman had two cryotubes bearing her identifier or code). The cryotubes were stored in the Hub-Kisangani at -80°C. Half of the cryotubes were sent to the large biochemistry laboratory of the University of Strasbourg's Faculty of Life Sciences, in collaboration with the laboratory of the Chemistry and Biochemistry Section of the University of Geneva's Faculty of Science, for comparative assays of trace elements. The sera were transported in a liquid nitrogen container to the laboratory at the University of Strasbourg.

The concentrations of trace elements in the sera were analysed using an inductively coupled plasma mass spectrophotometer (ICP-MS Agilent 7700X).

## 2.6. Data Processing and Analysis Plan

- 1) Manual data processing and categorisation;
- 2) Data analysis.

The data were entered on computer using Microsoft Excel 2021. Statistical

analysis of the data was carried out using R software version 4.3.0.

We used percentage calculations for qualitative variables, the mean, median, standard deviation, variance, minimum, maximum, first and third quartile, and mode for quantitative variables. We tested normality with the Kolmogorov-Smirnov test and we tested homogeneity of variances with the Levene test. We used the Kruskal Wallis test and the Wilcoxon Mann-Whitney test to compare the means between the different groups.

## 2.7. Operational Definitions

Socio-economic levels were determined using the household economic well-being index constructed by Tchamda and Nkabkob [16]. This index takes into account households' possession of certain durable goods and certain housing characteristics. For our study, in terms of household durables, we looked at whether or not households owned: television, radio, car, refrigerator and mobile phone; and in terms of housing characteristics, we asked questions about the availability of electricity, the supply of drinking water, the use of modern toilets, the construction of walls in durable materials and the use of embers or stoves as cooking fuel.

We first assigned a score to each of the goods or features of the dwelling: 1 for the "yes" response and 0 for the "no" response. We then assigned each household a total score corresponding to the sum of all the scores obtained for each property or housing feature. Households were ranked in ascending order of total score and divided into 5 categories known as quintiles: poorest (total score = 1 - 2), second poor (total score = 3 - 4), average (total score = 5 - 6), fourth rich (total score = 7 - 8) and richest (total score = 9 - 10).

-Civil servant in the private sector: employee in a non-governmental organization or independent contractor.

-Pregnant or gestating woman: A woman who is pregnant and whose diagnosis has been confirmed by a rapid urine pregnancy test and an ultrasound scan.

## 2.8. Ethical Considerations

The ethics committee of the University of Kisangani gave us its approval by letter No. of Approval: UNIKIS/CER/025/2022 of 26/12/2022 to begin with the collection of data. The research was carried out in accordance with the ethical standards set out in the 1964 Declaration of Helsinki and its subsequent amendments. All subjects gave written informed consent prior to inclusion in the study.

Anonymity was guaranteed during data collection, analysis and dissemination. Data was only collected once the woman had signed the informed consent form in the language she spoke.

## 3. Results

### 3.1. Socio-Demographic Characteristics of Respondents

**Table 1** shows that 75% of pregnant women were aged 20 - 34, compared with

**Table 1.** Socio-demographic characteristics of non-pregnant and pregnant women.

Features	Pregnancy		Total N = 1 189
	No, N = 598	Yes, N = 591	
<b>Age (years)</b>			
<20 years	112 (18.7%)	59 (10.0%)	171 (14.4%)
20 to 34 years old	385 (64.4%)	445 (75.3%)	830 (69.8%)
≥35 years	101 (16.9%)	87 (14.7%)	188 (15.8%)
<b>Address (Municipality)</b>			
Kabondo	85 (14.2%)	103 (17.4%)	188 (15.8%)
Kisangani	98 (16.4%)	46 (7.8%)	144 (12.1%)
Lubunga	101 (16.9%)	28 (4.7%)	129 (10.8%)
Makiso	119 (19.9%)	111 (18.8%)	230 (19.3%)
Mangobo	126 (21.1%)	166 (28.1%)	292 (24.6%)
Tshopo	69 (11.5%)	137 (23.2%)	206 (17.3%)
<b>Profession</b>			
State agent	29 (4.8%)	45 (7.6%)	74 (6.2%)
Retailer	54 (9.0%)	76 (12.9%)	130 (10.9%)
Seamstress	24 (4.0%)	33 (5.6%)	57 (4.8%)
Farmer	12 (2.0%)	23 (3.9%)	35 (2.9%)
Pupil	51 (8.5%)	16 (2.7%)	67 (5.6%)
Teacher	13 (2.2%)	23 (3.9%)	13 (2.9%)
Student	101 (16.9%)	27 (4.6%)	128 (10.8%)
Nurse	16 (2.7%)	22 (3.7%)	38 (3.2%)
Housewife	278 (46.5%)	308 (52.1%)	586 (49.3%)
Pharmacist	1 (0.2%)	0 (0.0%)	1 (0.1%)
Private sector employee	19 (3.2%)	18 (3.0%)	37 (3.1%)
<b>Marital status</b>			
Single	175 (29.3%)	74 (12.5%)	249 (20.9%)
Married	423 (70.7%)	517 (87.5%)	940 (79.1%)
<b>Level of education</b>			
Illiterate	3 (0.5%)	0 (0.0%)	3 (0.2%)
Primary	19 (3.2%)	17 (2.9%)	36 (3.0%)
Secondary	406 (67.9%)	446 (75.5%)	852 (71.7%)
University	170 (28.4%)	128 (21.7%)	298 (25.1%)
<b>Socio-economic level</b>			

**Continued**

The Poorest	14 (2.3%)	0 (0.0%)	14 (1.2%)
Poor	139 (23.2%)	25 (4.2%)	164 (13.8%)
Medium	243 (40.6%)	415 (70.2%)	658 (55.3%)
Rich	149 (24.9%)	139 (23.5%)	288 (24.2%)
The Richest	53 (8.9%)	12 (2.0%)	65 (5.5%)

64.4% of non-pregnant women, with an average age of  $26.3 \pm 6.7$  years. The extremes ages were 15 and 47 years. 52% of pregnant women were housewives compared with 45.6% of non-pregnant women; and 7% of pregnant women were state agent compared with 4.8% of non-pregnant women. 87% of pregnant women were married and 70.2% were of average socio-economic status.

### 3.2. Trace Element Status of All Respondents in General

**Table 2** shows that the mean calcium level was  $1.9031 \pm 0.49$  mmol/l, while the mean zinc level was  $11.1396 \pm 3.0874$  micromol/l. The medians for copper and selenium were 12.14 and 0.54 micromol/l. The magnesium mode was 0.87 mmol/l.

### 3.3. Trace Element Status in Pregnant Women

**Table 3** shows that the mean values for copper and magnesium in pregnant women were  $12.58 \pm 1.13$  micromol/l and  $1.03 \pm 1.03$  mmol/l respectively. The medians for calcium and zinc were 1.49 mmol/l and 8.42 micromol/l. The selenium

**Table 2.** Trace element status in women overall.

Trace elements	Average	Variance	Standard deviation	Minimum	P 25	Median	P 75	Maximum	Mode
Calcium (mmol/l)	1.9031	0.2428	0.4927	0.94	1.49	1.99	2.31	2.98	2.31
Copper ( $\mu$ mol/l)	13.0686	4.473	2.115	0.53	11.48	12.41	14.37	20.42	12.31
Magnesium (mmol/l)	0.9401	0.5905	0.7685	0.082	0.79	0.83	0.92	1.15	0.87
Selenium ( $\mu$ mol/l)	0.7306	4.4383	2.1067	0.2	0.46	0.54	0.76	1.26	0.54
Zinc (micromol/l)	11.1396	9.532	3.0874	2.41	8.42	9.71	13.79	16.43	7.49

**Table 3.** Trace element status in pregnant women.

Trace elements	Average	Variance	Standard deviation	Minimum	P 25	Median	P 75	Maximum	Mode
Calcium (mmol/l)	1.4896	0.0827	0.2876	0.94	1.24	1.49	1.69	2.22	1.54
Copper (micromol/l)	12.5796	1.2805	1.1316	11.04	11.71	12.33	13.24	14.99	12.31
Magnesium (mmol/l)	1.0334	1.0723	1.0355	0.082	0.82	0.87	0.94	1.05	0.87
Selenium (micromol/l)	0.4713	0.0044	0.0665	0.31	0.42	0.47	0.53	0.78	0.41
Zinc (micromol/l)	8.4199	0.3772	0.6141	7.29	7.87	8.42	8.81	12.72	7.49

mode was 0.41 micromol/l. The micronutrient deficiencies in pregnant women were calcium (0.94 - 2.22 mmol/l), selenium (0.31 - 0.78 micromol/l) and zinc (7.29 - 12.72 micromol/l).

### 3.4. Normality Tests

**Table 4** shows that the levels of all our trace elements do not have a normal distribution.

**Table 4.** Tests for normality in the distribution of trace elements.

	Pregnancy	Kolmogorov-Smirnov		Shapiro-Wilk	
		ddl	p-value	ddl	p-value
Calcium (mmol/l)	No	596	<0.001	596	<0.001
	Yes	591	<0.001	591	<0.001
Copper (micromol/l)	No	596	<0.001	596	<0.001
	Yes	591	<0.001	591	<0.001
Magnesium (mmol/l)	No	596	<0.001	596	<0.001
	Yes	591	<0.001	591	<0.001
Selenium (micromol/l)	No	596	<0.001	596	<0.001
	Yes	591	<0.001	591	<0.001
Zinc (micromol/l)	No	596	<0.001	596	<0.001
	Yes	591	<0.001	591	<0.001

## 4. Discussion

### Trace element status in pregnant women

In this research, we found that the different statuses of trace elements (Calcium, Copper, Magnesium, Selenium and Zinc) in pregnant females varied from 0.94 - 2.22 mmol/l for calcium, 11.04 - 14.99 micromol/l for copper, 0.082 - 1.05 mmol/l for magnesium, 0.31 - 0.78 micromol/l for selenium, and 7.29 - 12.72 micromol/l for zinc. Their averages were  $1.49 \pm 0.29$  mmol/l for calcium,  $12.58 \pm 1.13$  micromol/l for copper,  $1.03 \pm 1.04$  mmol/l for magnesium,  $0.47 \pm 0.07$  micromol/l for selenium, and  $8.42 \pm 0.61$  micromol/l for zinc.

Our results are lower than those observed by Zubero M.B. *et al.*, in Spanish pregnant women in 2022 [17], by Liu X. *et al.* in China [18], and Ejezie FE *et al.* in Nigeria [19].

However, the results of research carried out by Djangbletey R *et al.* in Ghana [20] and Zhang, Z *et al.* [21] in China are similar to our results.

Research carried out in developed countries is producing increasingly better results than that carried out in developing countries such as ours. The advantage of these countries is that there are dietary and nutritional controls and monitoring within their general population, most often not only for pregnant women but also for those who are not pregnant or of childbearing age, children and men, in order

to correct any macro- or micronutrient deficiencies observed in dietary intake before the onset of pregnancy, when the demand for trace elements will become increasingly important, and the appearance of pronounced deficiencies likely to give rise to serious nutritional diseases.

The differences in results can be attributed to factors that can influence serum levels of different trace elements, including pre-analytical factors such as alterations in blood pH, physical exercise, postural changes, increased ventilation and diurnal variations, and dietary habits [22]. Another factor that could have affected these different variations in trace elements would be proteinuria. Seasonal and climatic differences, as well as racial differences, could also explain the differences in results [23] [24].

Given that the level of trace element concentration during pregnancy is linked, on the one hand, to the concentration in the woman prior to pregnancy and, on the other hand, to the physiological changes associated with pregnancy, the low trace element statuses in our study are thought to result from the low level of trace elements found in women of childbearing age living in our town of Kisangani [25]. This could justify the inequalities in trace element values between our respondents and those in other countries or environments.

We agree with the data in the literature and the opinion of other researchers that the adequacy of micronutrient intake and the resulting blood concentration during pregnancy are also influenced by environmental, cultural and demographic variables, and it would be essential to understand at the level of the non-pregnant population which nutrients (or trace elements) from food intake are deficient or lacking and whether efforts are needed to optimise maternal nutritional intake, including food supplementation or fortification [26]. All these steps can only be taken with the clear understanding that differences in trace element concentration may be due to soil, geographical location, food preparation and processing, food accessibility, cultural practices, pollution or ethnic differences in body composition and genetics [27].

**Study limitations:** This research did not allow us to know the nutritional status of pregnant and non-pregnant women, and the daily intake of trace elements in each respondent, which would be useful for correcting deficient cases in the entire population of our research environment.

## 5. Conclusion

At the end of this research, we noted that the trace element status of pregnant women was lower than that of non-pregnant women. The micronutrient deficiency among pregnant women in Kisangani is a real problem revealed by this research, and should be of concern to all those involved in maternal and child health in our communities in order to find upstream solutions, especially in the management of micronutrient deficiencies within the population.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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