UNIVERSITY OF KINSHASA
Faculty of Medicine
School of Public Health

Micronutrients Deficiencies and Heavy Metals Exposure in Children Under Five Years in Popokabaka, The Democratic Republic of Congo

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M.D., M.P.H.

Dissertation for the Degree of Doctor of Philosophy (Ph.D.)
University of Kinshasa, DRC

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November 2023

REPUBLIQUE DEMOCRATIQUE DU CONGO
UNIVERSITE DE KINSHASA

Faculté de MEDECINE
B.P 834 KINSHASA XI
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....MICRONUTRIENTS DEFICIENCIES AND HEAVY METALS EXPOSURE IN CHILDREN UNDER FIVE YEARS IN KOLOKANGA, THE DEMOCRATIC REPUBLIC OF CONGO.....

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''Bring to us what we miss from our plates.''

Yannick, five years old

A Photo taken by Branly, in Ingasi village, Popokabaka
With the permission gathered from his mother during farming

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Contributing Institutions

This research was made possible under the GROWNUT 1 Project- *Growing up the partnership for higher education and research in Nutrition epidemiology- a* collaboration that included three academic institutions :

1. School of Public Health, Faculty of Medicine, University of Kinshasa, Kinshasa, the Democratic Republic of Congo

2. Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway

3. Centre of Rural Health, University of Kwazulu Natal, Kwazulu Natal, Durban, South Africa

Program Project: Grow Nut

Project number: QZA-0484, COG-13/0002

Funder: Norwegian agency of development (NORAD)

Dedication

To

My Father Kilola Mbunga and my Mother Molina Dinzenza,

To

My spouse Makongote Annick, and our daughters, Amelia, and Gabrielle Mbunga,

To

My Family Kilola's, Kilola Gloire, Kilola Grace, Kilola Marlyse, and Kilola Christie

I dedicate this thesis which I would not have completed
without their tremendous understanding, encouragement,
and support in the past few years

Acknowledgments

First and foremost, I am highly grateful to my supervisors, Professors Mapatano Mala Ali, Ingunn Engebretsen, and Tor Strand, who trusted in me more than myself for their valuable advice, continuous support, and patience during my Ph.D. study. They built my academic and research career with incredible competence and confidence.

I would also like to thank Professors Pierrot Lundimu and Elin Gjengedal for their technical support and guidance in my studies.

I would like to thank all the steering and review committees members of my Ph.D. project: Professors Mapatano Mala Ali, Patrick Kayembe, Fridolin Kodondi , Georges Mvumbi, Pierre Akilimali, Joel Tuakuila, Mulumba Nkuadi, Pelagie Babakazo, Adolphine Nkuadiolandio, and Paolo Bunga. It is their expertise that has shaped the success of my project.

I extend my gratitude to all staff members of the Department of Nutrition, the School of Public Health, the Faculty of Medicine, and the University of Kinshasa for their support and contribution in any steps of the realization of this Degree. Special thanks to these people who have encouraged and advised me so closely at different stage of this research: Professors Jean Marie Kayembe, Roger Mbungu, Erick Kamangu, Desire Mashinda, Joel Konde, Marie Onyamboko, Paul Lusamba, Didine Kaba, Muyer Telo, Pelagie Babakazo, Paulin Mutombo, Eric Mafuta, Aimee Lulebo, Dalau Nkamba, and Freddy Bangelesa. They involved themselves in my project to have it finally done.

I thank the GROWNUT Team, the Norwegian Agency for Development NORAD, and the Norwegian Embassy in DRC for the scholarship and all the support I received to make this study possible. Special thanks to Professors Thorkild Tylleskar, Anne hatloy, Ingunn Engebretsen, Christianne Horwood, Lyn Haskins, Bruno Sunguya, Paulin Mutombo, and Mapatano mala ali for your support and patience.

I am grateful to all my colleagues and friends at KSPH, let keep up this family spirit and sincerity in co-working. My research benefited a lot of such an ambiance and environnement. Special thanks to Bonsokie Marc, Benito Kazenza, Landry Egbende, Joel ndembe for your involvement to my field work and manuscripts preparation

During my PhD, I keep performing professional experiences at PATH Organization that help me to survive with my family. These wonderful people may found here my gratitude for their understanding and flexibility over all institution rules: Trad Hatton, Garyn Debondt, Musha Kalahizi, Nelly Moleka, and Fatou Fall.

Thanks to Mr. Philippe Lumeka for being a close model of excellence that continuously inspired me and supported my ambitions and faith in God's vision for this work.

Thanks go to our families Kilola, Mbunga, Ndombele and Makongote for the continued love and support we received so far. It is also because we are surrounded by protective lovers and good prayers that we have succeeded. Much love and thanks to my spouse Annick Makongote, for being a second and home supervisor to follow progress, support and encouraged me to do not give up. Respectful thanks to Molina Dizenza whose sacrifices find honor, for having not give up to invest in my education and studies since primary school.

Finally, I want to thank the Popokabaka community, which consented to share Information and data to serve the realization of this study. Nothing would erase the rural and community-based experience I got under their social warmth and welcome. To my research team in Popokabaka, who made a excellent and quality work from limited infrastructure conditions, Find here my gratitude.

Summary

Micronutrients Deficiencies and Heavy Metal Exposure in Children Underfive Years in Popokabaka, The Democratic Republic of Congo

Background: Micronutrient (vitamin or mineral) deficiencies are known to lead to poor growth, poor health, cognitive impairment, and a high risk of dying from infectious diseases. The Democratic Republic of Congo (DRC) is listed among countries with a high prevalence of Micronutrient Deficiencies (MND) based on proxy estimations. However, little is known about the burden and extent of specific MNDs. Popokabaka is an example of a rural context where communities experience poor food security conditions.

Objective: We aimed to measure the burden of micronutrient deficiencies, identify their Associated factors, and understand the food and nutrition context underlying MNDs to inform adapted actions and interventions in this area.

Methods: We designed a mixed-method study that included a biomarker survey, market surveys, and a qualitative case study. The Inductively Coupled Plasma-Mass Spectrometry, the electrochemiluminescence immunoassay, and the immuno-turbidimetry were used in laboratories to quantify, respectively, minerals, ferritin, and C-Reactive Protein in the serum of under-five children. Different household food indicators were collected, and food consumption of micronutrient-rich food was assessed. Food availability, cost, and client satisfaction were assessed at the local markets. Drivers of food production were also explored.

Results: Children were anemic but less iron-deficient. Anemia was highly linked to inflammatory diseases. Zinc and selenium deficiencies were severely prevalent. Arsenic was detected in almost all children and quantified in high levels in half of children. Arsenic levels were statistically and geographically negatively linked to inverse zinc, while mercury levels were positively linked to selenium. Animal sources of foods and sea foods were under-consumed in households, rare and expensive at markets, and livestock was not developed at all. Communities preferably grow cassava, maize, and groundnuts but shared insights and perceived drivers for diversified agriculture and improved livestock.

Conclusion: Not one but multiple MNDs should be expected to be severely prevalent and of urgent concern from Popokabaka and similar rural settings. The adapted theory of change we suggest in this thesis prioritizes the development of food-based approaches focused on livestock promotion for long stability and sustainability of good nutrition of children and all communities.

Keywords: Micronutrients deficiencies; Market analysis, Food insecurity, Children; Popokabaka

Résumé

Les Carences en Micronutriments et l'Exposition en Metaux Lourds chez l'Enfant de Moins de 5 ans à Popokabaka, en République Démocratique du Congo

Contexte: Les carences en micronutriments (vitamines ou sels minéraux) sont connues pour entraîner une mauvaise croissance, une mauvaise santé, une déficience cognitive et un risque élevé de mourir des maladies infectieuses. La République Démocratique du Congo (RDC) est classée parmi les pays ayant une prévalence élevée de carences en micronutriments sur base sur des indicateurs proxy: Cependant, on sait peu de choses sur l'ampleur et l'extension de carences de micronutriments spécifiques. Popokabaka est un exemple de contexte rural où les communautés éprouvent de difficultés averées de sécurité alimentaire.

Objectif: Nous avons mesuré l'ampleur des carences de certains micronutriments, identifié leurs facteurs associés et exploré le contexte d'alimentation et de nutrition sous-jacent aux MND pour orienter les actions et interventions adaptées dans ce domaine.

Méthodes: Nous avons réalisé une étude mixte qui comprenait une enquête de biomarqueurs chez l'enfant de moins cinq ans, des enquêtes de marché et une étude qualitative. La spectrométrie de masse plasma à couplage inductif, l'immunodosage électro-chimiluminescence et l'immuno turbidimétrie ont été utilisés au laboratoire pour quantifier, respectivement, les minéraux, la ferritine et la protéine c-réactive dans le sérum d'enfants de moins de cinq ans. Les mesures alimentaires au niveau de ménage ont été effectuées et la consommation alimentaire d'aliments riches en micronutriments a été évaluée. La disponibilité des aliments, les coûts et la satisfaction des clients ont été évalués sur les marchés locaux. Les facteurs influençant la production alimentaire ont également été explorés.

Résultats: Les enfants sont en majorité anémiques mais moins déficients en fer comme initialement attendu. L'anémie est fortement liée aux maladies inflammatoires. Les carences en zinc et en sélénium sont très prévalents. L'arsenic a été détecté chez presque tous les enfants et quantifié à des niveaux élevés dans le sérum d'un enfant sur deux. Le niveau d'Arsenic était statistiquement et géographiquement lié au zinc, tandis que le niveau de mercure était positivement lié au sélénium. Les sources animales d'aliments et les produits de mer étaient sous-consommées dans les ménages, rares et couteux sur les marchés. Les communautés cultivent de préférence le manioc, le maïs et les arachides, mais ont exprimé le besoin d'une agriculture diversifiée et un élevage moderne.

Conclusion: les carences en micronutriements sont hautement prévalents et de préoccupation urgente ? Popokabaka et des milieux ruraux similaires. Notre théorie adaptée du changement privilégie le développement d'interventions basées sur les aliments pour une longue stabilité et durabilité aux enfants et à toutes les communautés

Mots-clés: Déficiences en micronutriments; Enfants; Popokabaka

Mu Nkufi (Yaka Language)

Ukeya Kwa Binama Bia Madia Kwa Mwana Wena Mu Banda Mvula Tanu

Diambu diambu Dia Ku n'lambu wa Popokabaka, mu tsi ya impwanza kia Congo

Background (INZONZI) : ukeya mu binama bia madia(mfunda je n'sani) bizayekeni nde ibiau bikuma bia uyela kwa mbi, mavimpi ma mbi, igonza kia bâla lufwa mu bimbefo bia biniaka. Tsi Congo batanga yau gana kati kia zitsi zakonda je binama bia madia. Kinga mambu mavula tukondedi uzaya mumpila bita yandana unkonda kwa binama muna madia. Popokabaka yena mbandu muna bikanda bia magata bita zingila ye mpasi za madia.

Objective (NKUMA) : Twatesa ulututuka kwa ukeya kwa binama muna madia, twasengumuna buna biena ye twatoma landila n'samu wa madia, mudiambu twabaka mpangu zifwanakeni mu mambu ma madia.

Methods (TSALULU) : Twalongokedi nzingulu kwa mwana wena mu banda mvula tanu, ba ndonzi ba bifulu bia utekila balonguka nkalulu buna yena. Bafimpa mbote menga ye mamba m'a lutu lwa mwana wena mu banda mvula tanu m'uzaya nkaki ye madia ikotanga muna lutu lwandi. Tufweti landila ntumina za mambu m'udia muna zinzo buna masalamanga musiande badianga bima biena ye binama bia ngolo bia madia. Usonga madia, ntalu y'usumbila mau, ye ulungisa tsatu ya ba n'sumbi batesanga biau muna ma zandu mena mu magata. Bikuma bitwadisa usalula kwa bimenina bia madia, Twalongokedi mau

Results (ZI MVUTU) : Bana mu imvuka bankonda ye menga. Unkonda kwa menga kutwalanga bimbefo biabi bivimbisanga lutu. Ukeya kwa binama biabi biganganga ngolo mu lutu(zinc) kwavula.Muna kati kia mabuta madia ma n'suni je zitsema mena mavulako, mu itsunza bimonikinanga mani ntalu yingi bakeka biau. Batu batoma Zola badianga n'tombo, masidi ye nguba. Kansi basongedi tsatu y'ukuna bimenina bia mbandu zavula ye utsatsa bintwisi mumpila yampa.

Conclusion (TSUKA DIAMBU) : Ukeya kwa binama bia madia kwavula kwena: diambu Dia nkwanga Ku Popokabaka je bifulu biagika muna magata. Tsalulweto ita nonga Kaka mambu ma utoma utoma kwa madia mudiambu dia ukala te nzingulu yakaleya ye ukwamina mu luzingu lwa bana ye bibuka bia batu.

Keywords (N'GOGO MIA M'FUNU) : Ukeya kwa binama bia madia; Bana; Popokabaka.

List of Papers

The thesis is based on the following papers:

PAPER I (published)
Mbunga BK, Mapatano MA, Strand TA, Gjengedal ELF, Akilimali PZ, Engebretsen IMS. Prevalence of Anemia, Iron-Deficiency Anemia, and Associated Factors among Children Aged 1-5 Years in the Rural, Malaria-Endemic Setting of Popokabaka, Democratic Republic of Congo: A Cross-Sectional Study. Nutrients. 2021 Mar 21;13(3):1010. doi: 10.3390/nu13031010. PMID: 33801005; PMCID: PMC8003967.

PAPER II (published)
Mbunga BK, Engebretsen IMS, Strand TA, Gjengdal ELF, Akilimali PZ, Langfjord MM, Tugirimana PL, Mapatano MA. Distribution and Associated factors of Serum Zinc, Copper, and Selenium Levels among Children under Five Years from Popokabaka, Democratic Republic of Congo: A Cross-Sectional Study. Nutrients. 2022 Feb 6;14(3):683. doi: 10.3390/nu14030683. PMID: 35277041; PMCID: PMC8839910.

PAPER III (published)
Mbunga BK, Gjengedal ELF, Bangelesa F, Langfjord MM, Bosonkie MM, Strand TA, Mapatano MA, Engebretsen IMS. Heavy metals in children's blood from the rural region of Popokabaka, Democratic Republic of Congo: a cross-sectional study and spatial analysis. Sci Rep. 2022 Nov 3;12(1):18576. Doi: 10.1038/s41598-022-23332-4. PMID: 36329123; PMCID: PMC9633830.

PAPER IV (under review)
Mbunga BK, Kazenza B, Horwood C, Engebretsen IMS, Strand TA, Hatloy A, Connolly C and Mapatano MA. Associated factors of Micronutrient-Rich Food Consumption in the Rural Context of Popokabaka, the Democratic Republic of Congo: A Cross-Sectional Study.
Submitted to Public Health Nutrition Journal/Cambridge

PAPER V (under review)
Mbunga BK , Mapatano MA , Egbende L , Strand TA , Hatloy A , and Engebretsen IMS. An Example of a Convergent Mixed-Methods Analysis to Examine Food Security: 2 The Case of Popokabaka in the Democratic Republic of Congo
Submitted to Agriculture And Food Security Journal/ BMC Springer Nature

Dissertation at a glance

Study I	**Prevalence of Anemia, Iron-Deficiency Anemia, and Associated Factors among Children Aged 1–5 Years in the Rural, Malaria-Endemic Setting of Popokabaka, Democratic Republic of Congo: A Cross-Sectional Study**
Aim	To assess the prevalence of anemia, the role of Iron Deficiency using multiple parameters, and the factors associated with anemia in a malaria-endemic rural area.
Methods	Community-based cross-sectional study of 432 children aged 1–5 years from the Popokabaka, DRC. Hemoglobin and malaria prevalence were assessed using rapid finger-prick capillary blood testing in the field. Venous blood samples were analyzed for serum ferritin, serum iron, total iron-binding capacity, and C-reactive protein (CRP).
Results	Anemia was found in 294 out of 432 (68%) patients. Malaria was found in 375 out of 432 (87%), and ID in only 1.8% according to diagnosis by adjusted ferritin and 12.9% according to transferrin saturation. ID indicators were not significantly correlated with low hemoglobin levels. Malaria, fever, and CRP > 5 mg/L were major factors associated with anemia in Popokabaka.
Conclusions	Anemia control should focus on treating inflammatory conditions and infectious diseases among children in such settings.
Study II	**Distribution and Associated factors of serum zinc, copper, and selenium levels among children under five years from Popokabaka, Democratic Republic of Congo: A cross-sectional study**
Aim	To measure the distribution and Associated factors of serum zinc (Zn), copper (Cu), and selenium (Se) concentrations in a representative sample of children under five years old.
Methods	Community-based cross-sectional study in Popokabaka, DRC. Blood samples were drawn from 412 children. The serum concentrations of minerals were measured using inductively coupled plasma–mass spectrometry.
Results	The median concentrations (P25–P75) of Zn, Cu, and Se were 61.9 μg/dL (52.8–70.2), 145.5 (120.0–167.0) μg/dL and 5.3 (4.3–6.3) μg/dL. The CRP-adjusted prevalence of serum Se deficiency was 84.1% (95% confidence interval [CI] 81.4–87.0), and of Zn deficiency was 64.6% (95% CI 59.8–69.1%). Only a few children were Cu deficient [1.5% (0.6–3.2)]. There was evidence of inflammation (C-reactive protein, >5 mg/L) with a lower Se concentration and a higher Cu concentration. Furthermore, serum Se concentration was positively associated with linear growth. The average Cu/Zn molar ratio (2:1) was twice that recommended. Children in western Popokabaka had higher Zn and Se levels than their eastern neighbors.
Conclusions	Zinc and selenium deficiencies are common among children in Popokabaka and require attention and prioritization.
Study III	**Assessment of heavy metals in children's blood from the rural region of Popokabaka, Democratic Republic of Congo: a Cross-Sectional Study and Spatial Analysis**
Aim	To describe the distribution of four heavy metals [arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg)] in the serum blood of Popokabaka children DRC
Methods	The four metals were measured in 412 Children's blood samples aged 12 to 59 months using inductively coupled plasma-mass spectrometry (ICP-MS). Limits of detection (LoD) and quantification (LoQ) were set. Percentiles were reported. Statistical and geospatial bivariate analyses were performed to identify relationships with nutrition outcomes.
Results	Arsenic was quantified in 59.7%, while Cd, Hg, and Pb were quantified in less than 10%, all without toxicities. The arsenic level was negatively associated with the zinc level, while the Hg level was positively associated with the selenium level.
Conclusions	This common detection of As in children of Popokabaka requires attention, and urgent drinking water exploration and intervention for the profit of the Popokabaka community should be considered.

Study IV	**Associated factors of micronutrient-rich food consumption in the rural context of Popokabaka, the Democratic Republic of Congo: A cross-sectional study**
Aim	To assess food consumption patterns and Associated factors of the consumption frequency of micronutrient-rich foods among children under five years of age in Popokabaka, Democratic Republic of Congo (DRC).
Methods	A community cross-sectional study with a three-stage probabilistic sampling technique was conducted using a validated 16-item Food Frequency Questionnaire (FFQ), the Household Food Insecurity Access Score (HFIAS) questionnaire, the wealth index score based on household ownership, and other individual characteristics. Descriptive statistics and negative binomial regression models were performed. A total of 432 children and their mothers participated.
Results	Green leaves were the most commonly consumed food in Popokabaka (92%), an important source of micronutrients. Animal sources were consumed to a limited extent (insects, 10%; milk and dairy, 21%; and meat and chicken, 65%). As high as 88.4% of Popokabaka households experienced severe food insecurity access, while 40.7% of children had poor food consumption. Household food insecurity had a negative impact on the consumption of animal micronutrient-rich foods, whereas the wealth index and livestock activity were positively associated with consumption.
Conclusions	The present study highlights the importance of diet diversification and household food security, as well as the role of agricultural improvements as a potential contributor to household diet quality and micronutrient adequacy in children of Popokabaka.
Study V	**An example of Convergent Mixed-Methods Analysis in Examining Food Security: The Case of Popokabaka, the Democratic Republic of Congo**
Aim	To measure food security at four levels of the food chain and suggest integrative pathways of transformative impact on Hunger and malnutrition in Popokabaka, the Democratic Republic of Congo.
Methods	A convergent parallel mixed-methods study with four-level data sources was conducted in Popokabaka: a household food survey (using the Household Food Insecurity Access Scale (HFIAS), the Household dietary diversity score (HDDS), and the Food Consumption Score (FCS), a market food census (assessing food availability and cost per 100g), an exit food market survey (assessing food choice and client's satisfaction) and on-farm qualitative research with food producers (exploring challenges and opportunities).
Results	Popokabaka experienced severe food access insecurity (89%), poor food consumption (40.7%), and low dietary diversity (30.2%) at the household level. The quantitative findings at the household level were linked to market characteristics and farmer-reported themes under three pathways: poor diet quality, culturally grounded diet, and risk perception.
Conclusions	The focus should be on improving livestock development, developing adapted communications about nutrition to change established dietary habits and engaging the government and all stakeholders to empower local communities for improved food security.

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Acronyms

AGP : Alpha Glycoprotein
As : Arsenic
Cd : Cadmium
CRP : C Reactive Protein
Cu : Copper
DHS : Demographic and health survey
FCS : Food Consumption Score
FGD : Focus Group Discussion
HDDS : Household Dietary Diversity Score
HFIAS: Household food insecurity access score
Hg : Mercury
HH : Hidden Hunger
HHI : Hidden Hunger Index
ICP -MS:Inductively Coupled Plasma Mass Spectrometry
ID : Iron Deficiency
IDA : Iron Deficiency Anemia
IFPRI : International Food Policy Research Institute
MN : Micronutrient
MND : Micronutrient Deficiency
Pb : Lead
RBP : Retinol Binding Protein
RDC : The Republic Democratic of Congo
Se : Selenium
sTRF : Serum Transferrin Receptor
WHO : World Health Organization
Zn : Zinc

Chapter I. Introduction

1.1. Micronutrients during childhood age

Micronutrients (MN), including vitamins and minerals, are vital components of a high-quality diet that profoundly impact health. While they are only required in tiny quantities for the body, they are essential elements in many physiologic processes [1]. Consumption of a diverse range of nutrient-rich foods is usually sufficient to cover all needs of MN [2]. On the contrary, an inadequate intake or poor absorption of micronutrients can lead to *Micronutrient Deficiencies* (MND), leading to poor growth, poor health, mental impairment, and low productivity [3]. When this happens in early childhood, the consequences are severe, and the risk of dying from infectious diseases like diarrhea, measles, malaria, and pneumonia increases[2].

MND shows visible specific symptoms only when the deficit becomes severe (Hidden Hunger) [4]. For this reason, its history took time before attracting the attention of epidemiological research; the focus then was on severe protein malnutrition. MND started receiving serious attention from the international nutrition community in the mid-1980s and was raised at the International Conference on "Ending Hidden Hunger" in Montreal in 1991[5,4]. Then, the WHO supported decades of research assessment studies and recommended interventions to reverse MND prevalence.

Although any micronutrient lacking can cause health problems, the most damaging ones include Iron, Iodine, Zinc, Vitamin A, and folates [6]. They are, therefore, of public health importance globally. The International Food Policy Research Institute (IFPRI) estimated that half of the children aged 6 to 59 months suffer from one or more of those damaging MND and that one in three individuals is affected globally[7]. For the World Health Organization (WHO)[8], an estimated 250 million preschool children are vitamin A deficient worldwide. Concerning iodine, Anderson M. et al. [9] estimated that 29,8 % of individuals worldwide still suffer from low iodine intake. Furthermore, an estimated 17.3% of the world's population is at risk of inadequate zinc intake[10] while 25.0% have anemia attributable to iron deficiency globally [11].

In response to MND, cost-effective interventions have been implemented internationally, like vitamin A supplementation, iodine salt fortification, iron cereals fortification, and multiple micronutrient supplementation, each intervention showing a variable impact. However, the problem is still prevalent in developing

countries, especially in Sub-Saharan Africa, where regional prevalence and trends are expected to be the worst in the world based on proxy indicators [5]. Muthayya et al.estimated that of the 20 countries with the highest burden of MND globally, 18 were in sub-Saharan Africa with a high prevalence of stunting, associated with iron deficiency anemia (IDA) and vitamin A deficiency amongst preschool children [5].

1.2. Trends, prevalence, and successful interventions of key micronutrients

We present below a brief review of trends, prevalence, and successful interventions concerning the micronutrients of public health interest. Although the following review is drawn individually for each key micronutrient, research, and action, consider the coexistence of multiple MNDs at the individual or community level [12].

Iron is essential for motor and cognitive development and composes hemoglobin [13]. A low hemoglobin concentration (anemia) and a low iron storage capacity define iron deficiency anemia (IDA), which could lead to impaired cognitive and physical functionality and increased mortality risk in children [14]. In a systematic review of national surveys, Petry et al. [11] estimated the prevalence of IDA among preschool children at 25% worldwide, with a lower prevalence in developed than in developing countries. This finding is corroborated by Gupta et al.[15] in the United States, who reported an IDA prevalence of 1,1%, and by Harvey-Leeson S.[16] et al. in the DRC, who found an IDA prevalence of 20%. This disparity could be explained by a high incidence of other causes of iron depletion (Malaria, Hookworm infections) in children living in developing countries and inadequate iron intake. We can therefore understand that IDA is high in groups with inflammation/infection exposure. Thus, the IDA level needs to be adjusted for inflammation status (diseases, C reactive protein, AGP), which must be assessed. Cereal flour fortification and home-based sprinkling are the most recommended cost-effective community strategies in an area with a high prevalence of IDA.

Iodine is one of the most important minerals for brain and cognitive development [13]. its deficiency leads to a range of compromised physical and mental deficits called iodine deficiency disorders (IDD), including endemic goiter and cretinism [9]. The Lancet series estimated that populations with chronic Iodine deficiency have a 13.5-point reduction in IQ [12]. Meta-analyses[17,18] also proved that salt iodization has led to an increase in IQ and a significant decline in the prevalence of iodine deficiency

disorders, such as goiters. In a systematic review, Andersson et al. [9] reported that 39% of individuals (58 million) have inadequate iodine intake in Africa. The National Democratic Health Survey (DHS) report [19] in DRC indicates that 92.4 % of Households use adequate iodized salt.

Vitamin A is necessary to support healthy eyesight and immune system functions[13]. Vitamin A deficient children face an increased risk of blindness and death from infections such as measles and diarrhea [12] [13]. Globally, 1 in 3 preschool children is vitamin A deficient due to inadequate dietary intake [20]. Vitamin A supplementation of children 6-59 months is highly effective in reducing mortality from all causes in countries where vitamin A deficiency is a public health concern [20].

Zinc is the most ubiquitous element in the body and is involved in many enzymatic metabolisms. It promotes immunity, infection resistance, proper growth, and nervous system development[13]. Its deficiency is rapidly induced in humans because there is no known storage form in the tissue. On the other hand, there is a lack of obvious clinical signs of its deficiency [13] [21] [22]. For the International Zinc Nutrition Consultative Group (IZiNCG), stunting (short height at a specific age) can be used as a proxy indicator that reveals the presence of zinc deficiency in children [23]. Reviewing national biochemical surveys in Latin America and the Caribbean, Celdiel et al. [24] reported that Zinc deficiency ranged from 19.1% to 56.3% in children under six. Similar findings also are reported in sub-Saharan Africa: in 2016, Galetti et Al. [25] reported that 45,7% of preschool Beninese children were zinc deficient; Motadi S. A. et al. 24 in the rural area of Limpopo in South Africa found that 42.6% of children aged 3 to 5 years were zinc deficient. These data suggest that one in two preschool children could be zinc deficient. Like IDA, inflammation or infection status like diarrhea may also expose to zinc deficiency by loss. Tor et Al. [26] found that having dysentery and an elevated plasma C-reactive protein concentration were also independently associated with lower plasma zinc.

As diet is the primary micronutrient source for humans, food context and dietary patterns must be explored to understand pathways underlying deficiencies before averting good strategies and adapted actions to alleviate MND in a specific area.

1.3. Nutrition and food security in the Popokabaka context: the Cassava Diet

Food context and Dietary patterns in the genesis of the MNDs are not sufficiently described in the literature. Many studies focus only on nutrient levels in the blood and suggest general strategies that use micronutrients as treatment, like nutrient supplementation. However, this is usually a short-term solution. FAO strongly emphasizes that food-based approaches, including food production, dietary diversification, and food fortification, are sustainable strategies for improving the micronutrient status of the population.

In tropical Sub-Sahara, cassava is to African communities as rice is to Asians, or wheat and potatoes are to Europeans. Its root represents an excellent energy food source, rich in carbohydrates but poor in protein, fat, vitamins, and minerals. For instance, 100 grams of cassava roots contain less than 1 μg of β-carotene, 3 μg of zinc, and 4 μg of iron, which are not enough to cover daily human needs. Its green leaves are cooked as vegetables and also contain small amounts of vitamins and minerals [27]. Using a 24H recall, Gegios et al. [27] found that children in Nigeria and Kenya who consumed cassava as a staple food are at risk of inadequate zinc, iron, and vitamin A intake.

In addition, cassava also contains antinutrients and toxic substances that may interfere with the digestibility and absorption of some MN: Cyanide, phytates, tannins, and oxalates [28]. Cyanide is the most toxic factor that, in long-term intake, could cause severe health problems such as Konzo (irreversible spastic paraparesis) if not well processed [29] and goiter and cretinism by combining with low iodine intake [30]. Phytate (inositol hexakisphosphate) is another compound in high abundance in cassava, with approximately 624 mg/100 g in roots (Marfo and others 1990). it can bind cations such as magnesium, calcium, iron, and zinc and can, therefore, interfere with mineral absorption and utilization. Tannins (Polyphenol) can form insoluble complexes with divalent ions such as iron, zinc, and copper, limiting their absorption. Oxalates are antinutrients affecting calcium and magnesium bioavailability and form complexes with proteins. Again, farmers in some regions like Popokabaka prefer the Bitter cassava varieties because of their excellent resistance and availability in soil scarcity. However, this variety is highly concentrated in the antinutrients listed above.

Moreover, the soil is another important context when establishing micronutrients for a specific population. Every food from the soil inherits its composition. It could be possible that crops, animals, and even humans would be deficient in micronutrient deficient soil.

Adversely, if that soil contains heavy metals known to complex and limit micronutrient absorption, communities may also have abnormal heavy metal levels [31]. Bortey-Sam et al. [31], in Ghana, assessed the extent of heavy metals accumulation from soils to foodstuffs commonly grown in agricultural areas and evaluated its potential human health risks to residents in Tarkwa, Ghana. They reported considerable risk values of lead (Pb) and Nickel (Ni) in a population where malnutrition is of concern. In RDC, Bumoko et al.[32], in serum of konzo children, detected neurotoxic lead, mercury, manganese, cadmium, and cobalt compared to non-konzo children.
Children living in an area with soil scarcity and a monotonous cassava diet may be exposed to significant MND and health risks that need attention.

1.4. Research gaps

Little is known about MNDs in the DRC: The last DHS [19] found that 60% of 6-59 months old children were anemic. The survey does not, however, report on any minerals or vitamin deficiencies. Anational survey [33] conducted in 1998 established that 61% of preschool children were deficient in Vitamin A. In 2016, Harvey-Leeson S. et al. [16] searched micronutrient status in some Kivu and Kongo Central Provinces health zones and found that zinc deficiency was the most prevalent MND. They reported that Inflammation-adjusted zinc deficiency was 24% among 6 to 59 months and found an unexpectedly low prevalence of vitamin A, Iron deficiency anemia, and vitamin B12 [16].

However, the situation might be different and severe in other parts of the country. For instance, in regions like Popokabaka, where communities face food insecurity, limited nutrient-rich food availability, low access to dairy, meat, sea products, and preferably crop bitter cassava as a staple food source [32], it can be hypothesized that children are at higher risk of MND and health problems.

Thus, reliable community-based estimates of MN status and dietary patterns will help to contextualize MND and inform on the risk of living in such an area and strategies. This will allow the development of appropriate and cost-effective actions

to alleviate MND. The present project aimed to generate integrated and comprehensive knowledge that can improve local policymaking against MND in the context of Popokabaka.

1.5. conceptual Model frameworks

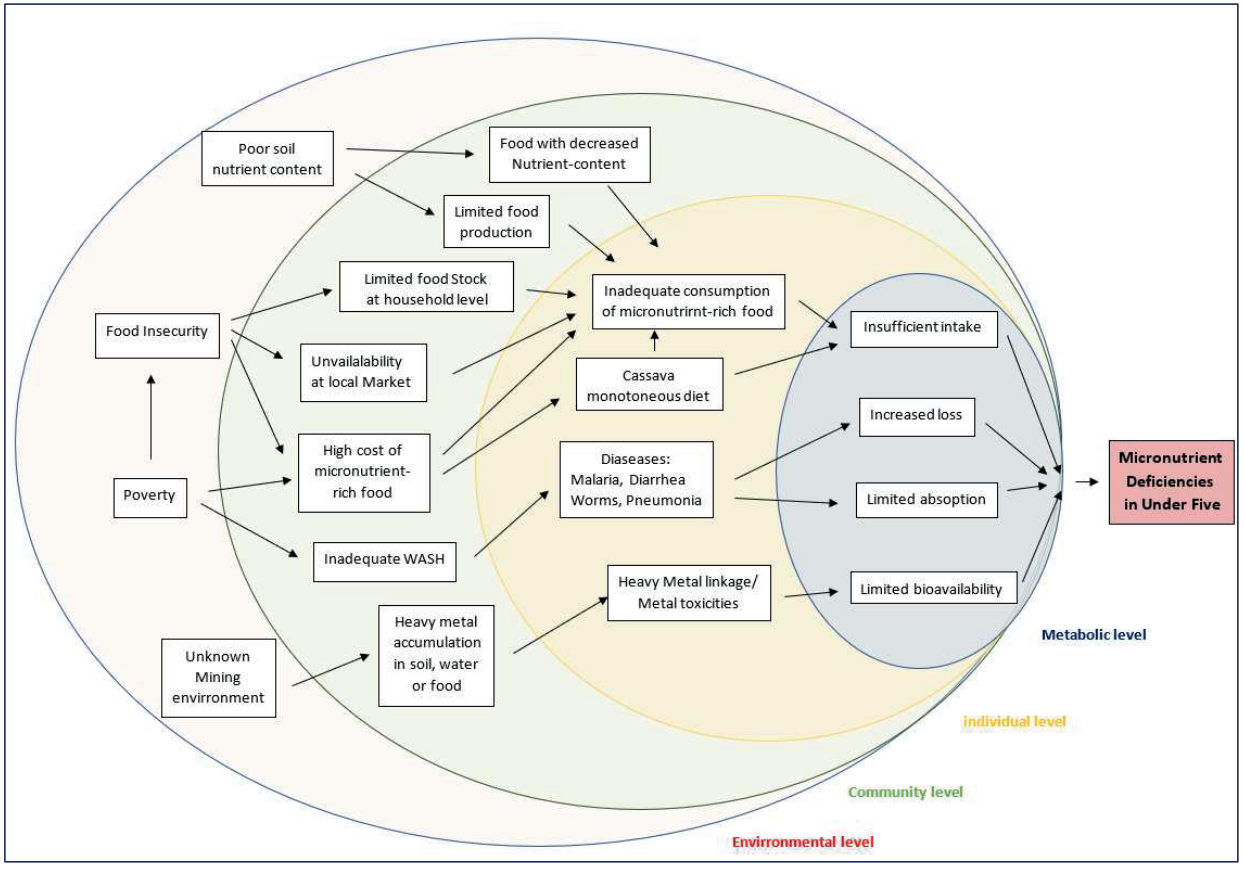

Figure 1. Socio-ecological framework hypothesizing Associated factors of Micronutrients deficiencies among Popokabaka under-five children

Chapter II. Objectives

1.1. Primary objective

To measure the burden and identify Associated factors of micronutrient deficiencies (Iron, Zinc, Selenium, copper) in the serum blood of children aged 1-5 years and assess the risk of heavy metals toxicity under the Food and nutrition environmental context of Popokabaka community, DRC.

1.2. Specific Objectives (SO)

SO1: To assess the prevalence of anemia, the role of Iron Deficiency using multiple parameters, and the factors associated with anemia among children living in the malaria-endemic rural Popokabaka

SO2: To measure the distribution and identify Associated factors of serum zinc (Zn), copper (Cu), and selenium (Se) concentrations in children under five in Popokabaka

SO3: To describe the distribution of four toxic elements [arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg)] in the serum blood of Popokabaka children DRC

SO4: To assess food consumption patterns and Associated factors of the consumption frequency of micronutrient-rich foods among children under five in Popokabaka

SO5: To examine and explore the comprehensive food security context that underlines micronutrient deficiencies in Popokabaka

Chapter III. Methods

3.1. Study design

The present study was set as a ***parallel convergent mixed-method design*** ([34]) to integrate quantitative and qualitative approaches carried out during the same period (April – August 2019) within the same study area. This design encompassed three components:

- The Fist component consisted of a "***biomarker cross-sectional study***" at the household level with a representative sample of children 1-5 years old to determine the prevalence and associated factors of MND and heavy element toxicity
- The Second component consisted of an "***availability and cost Market survey***" of micronutrient-rich foods, natural or fortified, sold at local markets.
- The third component consisted of a "***qualitative case study***" with local farmers exploring crop production of micronutrient-rich foods and soil amelioration.

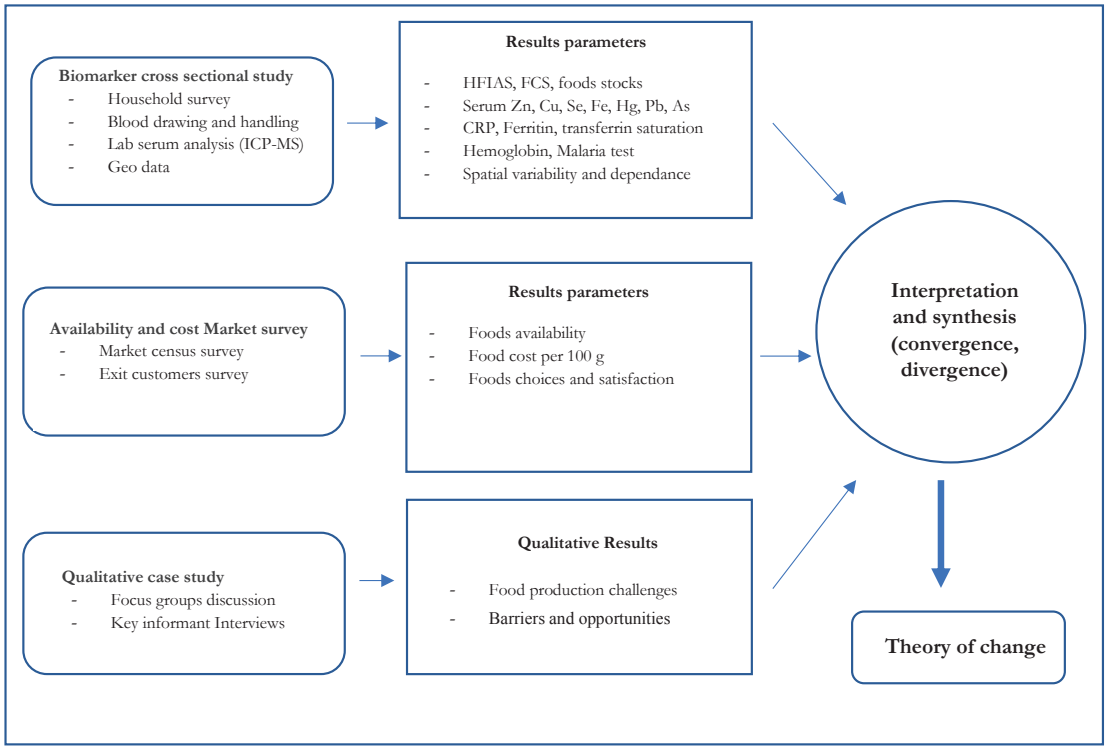

Figure 2. Study design flow chart- Micronutrient deficiencies in Popokabaka

3.2. Study setting

The study was conducted in the Popokabaka Health Zone, one of the 516 Health Zones (HZ) of the DRC, located in Kwango Province. Popokabaka Health Zone is an entirely rural region that extends over 6949 km2 (5°22'49.26" S –5°22'49.26" S, 16°20'26.16" E –16°20'26.16" E). The region is known for its poor soil and limited geographical access. The soil is sandy and almost arid and does not permit the growing of a wide variety of crops. Agriculture, which is not diversified, remains the primary source of income for the community. People prefer to grow cassava plants because of their resistance to soil drought, marketable roots, and culturally accepted leaves. They consume their roots and leaves as staple foods.

Raising livestock, poultry, and fishing are not practiced as much as cassava growing. People avoid fishing due to their fear of crocodiles in the Kwango River, which, divides the Popokabaka region into two. This river takes its source in Angola and crosses the diamond mining region of Kayemba. People in Popokabaka drink untreated water from groundwater sources and live under houses built on earth materials.

There are six official local food markets in Popokabaka, which take place twice a week on regular and set days. Three markets are located on the western side of the Kwango River (Ngasa, Ibuka, and Kisoma), and three are on the eastern side of the river (Citepopo, Imbela, and Kiamfu Kinzadi). Usually, people walk several kilometers to reach the official local market to sell their agricultural production or stock household foods.

Konzo, a food-based neurotoxic motor disease, is prevalent in the region, mainly affecting women and children [35–37]. Malnutrition and micronutrient deficiencies are also common among children under five [38–40]. Several organizations, such as the Food and Agriculture Organization (FAO) [41], CARITAS [42], Congodorpen [43], and ISCO [44], have led projects assisting farmers and providing equipment to support better agricultural production over the last ten years.

Although the MND burden is unknown in the region, nutrients specific interventions are ongoing: (Vitamin A supplementation, salt iodization, and iron fortification) are being promoted nationally and locally to prevent malnutrition.

3.3. Study Population and Sampling

A. Biomarker cross-sectional study

The survey concerned households having children aged 12 to 59 months old living with parents who had given written consent to participate in the study. Only one child was assessed in each sampled household. A child (and his household) was excluded if the parents refused blood drawing from the child or if the child was hospitalized for any diseases within the two previous weeks. The minimal sample size was estimated using Fischer's binomial formula to estimate the proportion in a community-based survey:

$$n = 1.96^2 x \frac{p(1-p)}{d^2} \; x \; \frac{Deff}{v}$$ where

- p is the expected proportion (or previous prevalence) of the MND which has the highest variance $p(1\text{-}p)$,
- d is the level of precision for a suitable confidence interval,
- *Deff* is the design effect for adjusting the cluster sampling error effect,
- And v is the response rate for adjusting the non-respondent error.

We considered the proportion value of anemia p= 0.59 for its huge maximum variance over all MND p x (1-p) =0.24 with a precision d of **0.075** and a design effect of 1,5 given the financial and logistical constraints associated with collecting and analyzing such high-quality biomarkers.

$$n = 1.96^2 x \frac{(0.59)(0.41)}{(0.075)^2} x \frac{(1.5)}{(0.80)} = 307 \; children \; or \; Households$$

Children were selected by using a probabilistic three-stage cluster sampling plan:

- In the first stage, we randomly selected five clusters (health areas) among the nine accessible areas through a probability-proportion-to-size technique. The term "accessible" considered the time constraints for collecting venous blood samples from the field and being able to process them within three hours at the Popokabaka Hospital. Only 9 of the 25 health areas fit this technical constraint.
- In the second stage, we randomly selected three villages in each cluster from the list of villages per health area the health zone manager provided us.
- In the last stage, an equal number of 30 households having children aged 12–59 months old were systematically selected from a detailed list pre-established by community workers in each village. Only one child from each household was selected and assessed.

B. Market survey

Four markets (Cite Popo, Imbela, Ngasa, and Ibuka) from the six official markets in Popokabaka were randomly selected and visited. All vendors (precisely 523) found on that visit day were

interviewed. All available food types were recorded. Weight and price were captured. (***the market census***)

Parallely, at the exit points of these markets, clients (precisely 147) that came out purchasing foods were selected based on a systematic sampling with a sampling interval of 1 and interviewed on satisfaction and affordability (***the exit interview***).

C. Participatory qualitative study

The focus group discussion (FGD) participants were smallholder farmers affiliated with a cooperative farmers' organization possessing crops/livestock/fishing farms. They were purposively selected from different clusters of markets. A total of 48 participants were purposively selected to form Six FGDs for theoretical data saturation.

The Key informant interviewees were community and civil society leaders involved in agricultural organizations. Seven key informants were purposively selected.

3.4. Data collection

A. Biomarker cross-sectional study

Data were collected using a questionnaire completed on tablet computers using the Survey CTO collect' application. The questionnaire consisted of eight modules:

- Household characteristics;
- Water, hygiene, and sanitation (wash);
- Household food security (household food insecurity access scale-HFIAS);
- Child health history;
- Infant and young children feeding practices;
- Anthropometric measures;
- Dietary practices (24 h recall and food frequency);
- And biochemical sampling.

Data collection took two consecutive days in each cluster, with three teams working in parallel for each of the three selected villages of the cluster. A team comprising three data collectors and two phlebotomists was formed.

On the first day, the data collectors visited the households, obtained consent forms, and completed the surveys for each child selected in the village. The biological mother or caretaker responded to the questionnaire, and anthropometric measurements were taken from the child. When finished, the personnel provided a card to the mother with the correct personal identifier for blood collection the next day.

On the second day, the phlebotomists went to the same villages at a specific identified location (the Health Center or another appropriate place) from 7 to 9 am for blood collection and hemoglobin and malaria rapid testing. The mothers were then invited to bring the children and the cards for blood collection.

The data collectors were trained by the investigator on the survey questionnaire and interview techniques, and the phlebotomists were trained by a lab expert on appropriate standard operating procedures (SOPs) and blood sample management.

B. Market survey

From the Market census, we recorded details of all foods sold (food items' names, types of food groups, quality, weight, and price) and interviewed vendors on the availability, volatility, and seasonality. Every food item was weighed using standardized kitchen scales to estimate the cost per 100 grams of net weight.

From exit interviews, Information on food choices, accessibility, affordability, and satisfaction was collected.

Again data were collected using structured questionnaires on tablet computers using the Survey CTO collect' application.

C. Participatory qualitative study

Qualitative data were gathered by using a discussion or interview guide, respectively. Discussions were conducted in the Lingala language. The principal investigator led all FGDs, and KII and was assisted by a note-taker. All discussions were recorded using a high-quality voice recorder after permission had been obtained from the participants.

3.5. Data management

A. Biomarker cross-sectional study

The phlebotomist first performed a capillary finger-prick test for hemoglobin (Hgb) assessment (Hemocue 301) and a rapid test for malaria in the field. Additionally, he collected up to 6 mL of venous blood from the child using trace-element-free serum BD vacutainers (BD-368380) and powder-free sterile disposable gloves. The Tourniquet application took a maximum of one minute. The collected blood was allowed to clot for at least 30 min in the field at room temperature and transported to the Popokabaka Hospital within 3 hours. There, it was centrifuged, and the supernatant was separated at 2300 rpm for 10 minutes using a Hettich Rotor 32A centrifuge

(Tuttlingen, Germany). The serum was aliquoted into two polypropylene vials: 0.5 mL tri-coded FluidX, Brooks Life Science, and 2 mL Sarstedt vials.

All the vials were immediately stored in a –40 °C freezer that worked nonstop on solar energy during the day and on a generator overnight in Popokabaka Hospital. When the survey was complete, the samples were transported from Popokabaka to Kinshasa (a 12 hours vehicle trip) and stored in liquid nitrogen. Then, every sample was stored at –80 °C in an ultra-low freezer at the Kinshasa School of Public Health for a week before being shipped on dry ice to Norway.

All the 0.5 mL vials were sent to Haukeland University Hospital (Bergen, Norway) for the analysis of serum ferritin (using electrochemiluminescence immunoassay (ECLIA)), C-reactive protein (S-CRP) (using the immunoturbimetry method), and total iron-binding capacity (S-TIBC, using the Berekna equation calculation: s-TIBC = s-Transferrin * 25,1). Transferrin saturation (TSAT), expressed as a percentage, was then calculated as the value of serum iron divided by the TIBC.

Two-milliliter vials were sent to the Norwegian University of Life Sciences (Ås, Norway) for the analysis of serum iron, zinc, copper, and selenium (using the Agilent 8900 Triple Quadrupole inductively coupled plasma mass spectrometer (ICP-MS)).

For quality control and accuracy during the lab analysis, samples were prepared together with ten blank vials that were taken through the measurement procedure. The limit of detection and limit of quantification ratio LoD/LoQ were determined in mg/L as Fe (0.030/0.11), Cu (0.0010/0.0049), Zn (0.060/0.20), and Se (0.0008/0.0027), As (0.0002/0.00055), Cd (0.000006/0.000019), Hg (0.0002/0.00083) and Pb (0.0006/0.0021).

Anemia was defined as Hgb levels <11 g/dL, and Iron Deficiency (ID) was set for serum ferritin concentrations <12 μg/L in the absence of inflammation or for transferrin saturation <20% regardless of inflammation status [45].

Deficiencies were set serum Cu of <80 μg/dL, Zn of <65 μg/dL [46], and Se of <7.0 μg/dL [47].

To account for inflammation [48], the regression-correction approach developed by BRINDA was used for minerals that correlated with CRP using the following equation: Adjusted mineral = unadjusted mineral – β (CRPobs–CRPref). First, we defined internal reference values for inflammatory markers (CRPref) as the tenth percentile [49]. Then, the regression coefficient (β) for the association was estimated between CRP and each mineral value using univariable linear regression models, with minerals as dependent variables.

A child was said to have malaria when there was a positive result in the rapid malaria test for Plasmodium falciparum.

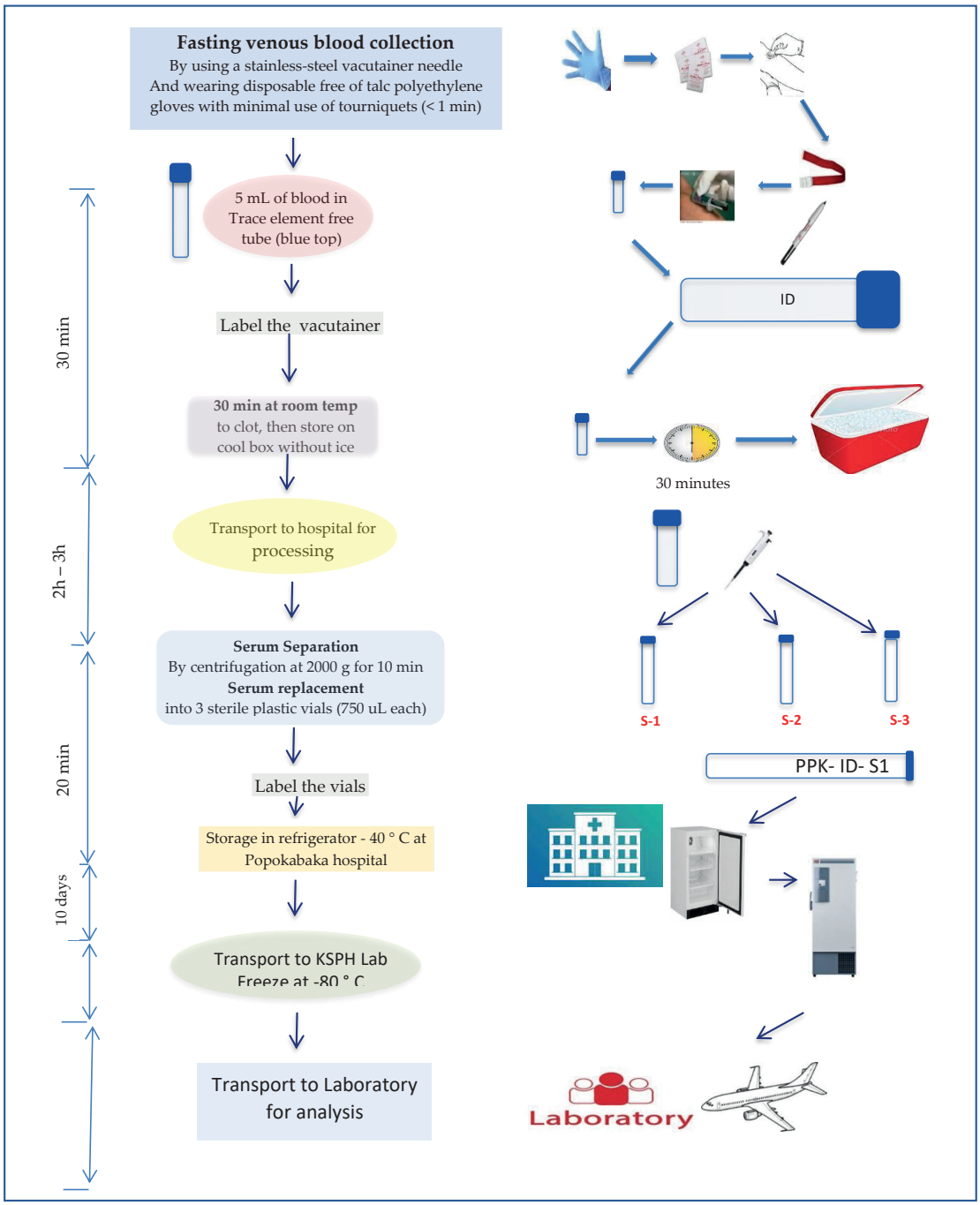

Figure 3 Standards operating procedures (SOP) for handling blood samples

Anthropometric indices, including the weight for height, height for age, weight for age, and mid-upper-arm circumference for age, and their Z-scores, were calculated using the WHO Anthro software. Wasting was defined as a weight-for-height Z-score (WHZ) < −2, stunting was defined as a height-for-age Z-score < −2, and underweight was defined as a weight-for-age Z-score < −2.

For each household, Wealth index quintiles were generated using principal component analysis on ownership variables in the household. Dietary patterns were estimated using three indicators: the Food Consumption Score (FCS), the Household Food Insecurity Access Score (HFIAS), and the Household Dietary Diversity Score (HDDS) [50]. The FCS classified households as having 'poor' (FCS ≤28), 'borderline' (FCS between 29 and 41), and 'acceptable' (FCS ≥42) consumption. The HFIAS classified households as food secure (HFIAS 1–5), mildly food insecure (HFIAS 6–10), moderately food insecure (HFIAS 11–15), and severely food insecure (HFIAS ≥15). The HDDS was used to classify households as having diversified (HDDS <4) and non-diversified (HDDS ≥ 4) diets.

B. Data management for the qualitative study

All digital audio recordings were transcribed verbatim in French. Thematic analysis was used, as recommended by Braun and Clarke [51]. Data reduction and preparation were processed using Atlas.ti 22.0 software. In total, 13 transcripts were read and coded by following two patterns (barriers and opportunities). After data reduction, 114 quotes were identified in the transcripts and clustered around 15 emergent sub-themes for barriers and 13 emergent sub-themes for opportunities. Sub-themes were then grouped into larger themes of barriers and opportunities. Quotations, codes, and themes were listed in a Microsoft Excel matrix and translated into English at this stage. Finally, the themes were discussed with the remaining coauthors of this study to ensure conformability [52].

3.6. Statistical analysis

All the quantitative data were analyzed using STATA 16.0.

We described important variables through univariate analysis: The median and 25th–75th percentiles are reported for continuous variables that were not normally distributed, while the mean (SD) is used for continuous variables that were normally distributed. Frequencies are used to describe categorical data. The confidence intervals (CIs) for prevalence were calculated using a normal Z-test.

The bivariate analysis also was performed: the Pearson chi-squared test was used to check for the association between binary outcomes and other categorical covariates. The Spearman correlation coefficients were used to describe the relationships between the continuous variables and continuous outcomes.

Different multivariate models were built depending on the nature of outcomes considered: Simple linear regression to adjust ferritin, zinc, and selenium concentrations for inflammation (C reactive Protein), Multiple linear regression to identify Associated factors for serum Zn, Se, and Cu levels, Multiple logistic regression to identify factors associated with anemia, Negative binomial regression to search for Associated factors of discrete outcomes ables (frequency of consumption of micronutrient-rich foods).

All regression models were constructed by a forward stepwise selection approach with covariate inclusion probability (p-value of crude OR) < 0.20. Multicollinearity was checked for all the covariates included in the regression model, and the Variance inflation factor (VIF) was calculated. All the statistical analyses were performed at a 0.05 level of confidence.

We also performed a spatial analysis concerning the association and dependence between heavy metals (As Hg) with a proven statistical association with essential minerals(Zn and Se). We used the inverse distance weighted spatial interpolation approach to map the distribution of these minerals/heavy metals [53,54]. Spatial statistics were applied using Lee's L bivariate spatial autocorrelation test [55] to capture the spatial association between heavy metals and other essential minerals. This test integrates information from Pearson's r (spatial bivariate association measure) and Moran's I (univariate spatial association measure). We further computed the local bivariate spatial association to assess the individual area's contribution to the global L and the spatial bivariate heterogeneity. Analysis was conducted in R (R Core Team, 2014), and maps were produced using the package leaflet.

3.7. Ethics consideration

The present study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Regional Norwegian Committee for Medical and health research ethics (REK). Committee *(ref: 2018/1420/REK vest, date: 30.11.2018)* and the Kinshasa School of Public Health ethical committee *(ref: ESP/CE/002/2019, date: 28.01. 2019).* Other authorizations and clearance were obtained from the local administrative and health authorities before any field work (households, markets, or food production places).

For the Biomarker survey, Mothers (caregivers or both parents if present) provided written consent for their child. They were informed of the completed steps and processes of the study. No child and no household were surveyed without written consent. When gathering the consent, we emphasized the voluntary character of their participation. Mothers were free to refuse to answer any question that would have made them and their children uncomfortable and choose to end the interview at any time. Before enrolling, they should consent to all steps, including a blood draw. We emphasized confidentiality. No name or other identifying data was attached to the child's

blood sample or the survey questionnaire. A study code identified the household and the child. The blood collection procedure may cause some discomfort and slight bruising or, very rarely, an infection at the site of the needle poke. After the blood draws, we were immediately given a bandage to cover the spot where the blood was taken. Finger drops were used for anemia testing, and the result was immediately told to the mother. Every severely anemic child was excluded from the present study and referred to the health center for treatment. The mother and her child received a package for participating in the study. In addition to this benefit, a rapid malaria test was done on the blood collected. The study was to provide health centers with medications for those found with malaria. Children enrolled in the study had the same advantages and were treated identically. They were selected randomly by using a probability sampling technique.

At the market level, authorization was gathered from local Managers before visiting. Written informed consent was also obtained from vendors in the market. As the analysis unit is the food product, no ethical issues were in recording the product's name. However, each vendor was assigned a unique identifier after a market mapping to avoid redundancy. There was no human risk in this part of the study. We explained to the vendor that the benefit for them is to contribute to this comprehensive MND Study that may lead to more availability interventions of nutrient foods to be sold. No money or incentives was given in the context of this study. Nevertheless, we ensured that no seller should be interviewed as he is busy selling.

For the qualitative study, oral informed consent was obtained from participants in the FGD before starting any discussion. We also got permission from them to record the discussion. The participants were informed that they could leave the discussion without penalty. The assurance was also given for confidentiality in data management and reporting quotes. Participants in FGD took time away from the woman's family or work, around two hours; therefore, study participants were compensated for their participation in the survey. Participants in the qualitative study received incentives in nature.

Chapter IV. Summary of Results

4.1. General Characteristics of the study population

Table 1 shows the general characteristics of the children included in this study.

Table 1. General characteristics of children aged 1–5 years in Popokabaka, n(%).

		n (%)
Age (months)	*Median (P25–P75)*	32 (22–43)
Age Groups (months)		
12–23		124 (28.7)
24–35		120 (27.8)
36–47		116 (26.8)
48–59		72 (16.7)
Gender		
Boys		224 (51.8)
Girls		208 (48.2)
Having Diarrhea in the last two weeks		78 (18.1)
Having Bloody stools in the last two weeks		18 (4.17)
Having a Fever in the last two weeks		249 (57.6)
Having a Cough in the last two weeks		141 (32.6)
Uptaking Zinc tablets in the last two weeks		23 (5.3)
Uptaking Vitamin A supplements in the last six months		232 (53.7)
Uptaking Deworming tablets in the last six months		306 (70.8)
Uptaking Iron supplements in the last three months		154 (35.6)
Sleeping under mosquito nets		260 (60.2)
CRP > 5 mg /L		207 (49.4)

The serum was collected from 412 children aged 1–5 years. The boy/girl ratio was 1:1, and their median (P25–P75) age was 32 (22–43) months. From the two preceding weeks, half of the children had experienced fever (57.6%), confirmed by an elevated CRP level in almost 49% of children. Children also experienced diarrhea (18.1%) and cough (32.6%) in the two preceding weeks.

Table 1 also reveals the existence of hospital-based childhood health and anemia-control national strategies implemented in Popokabaka, with, however, sub-optimal coverage: two weeks before our study initiation, Children had received nutritional supplements, such as Zn (5.3%) or iron supplements or syrups (35.6%).

Community-based interventions also exist as nutrition campaigns: almost seven out of ten children had benefitted from deworming in the previous six months, while half had received vitamin A in the previous six months.

4.2. Study outcomes distribution

Table 2 summarises the prevalence of nutrition outcomes among under five children of Popokabaka.

Table 2. Distribution of nutrition outcomes from the biomarker survey

	N	Proportion	CI95%
Stunting, as *HAZ<-2SD*	242/432	56.0	51.3- 66
Wasting, as *WHZ< - 2SD*	48/432	11.1	8.4 14.1
Anemia, *as CRP-adjusted*	294/432	68.1	64.0-72.0
Malaria *(positive rapid P.Falciparum test)*	375/432	86.8	83.6-90.0
Iron deficiency, *by CRP- adjusted ferritin*	7/400	1.8	0.5–3.0
Iron deficiency, *by transferrin saturation*	55/412	12.9	9.6-16.1
Zn deficiency, *as CRP-adjusted*	266/412	64.6	59.8- 69.0
Se deficiency, *as CRP-adjusted*	358/412	84.1	81.4-87.0
Cu deficiency, *as CRP-adjusted*	6/412	1.5	0.6-3.2
As detection, *as LoD As= 0.2 ug/L*	396/412	96.6	93.3 97.6
Hg detection, *as LoD Hg= 0.2 ug/L*	272/412	66.0	61.3 70.5
Pb detection, *as LoD Pb= 0.6 ug/L*	54/412	13.1	10.1 16.6
Cd detection, *as LoD Cd= 0.006 ug/L*	80/412	19.4	15.8 23.5
Food Consumption Score (FCS)			
Adequate	80	18.6	15.1-22.4
Limited	176	40.7	36.2-45.4
Poor	176	40.7	36.2-45.4
Household Dietary Diversity Score (HDDS)			
Diversified	133	30.8	26.6-35.3
Non-diversified	299	69.2	64.7-73.4
Household Food Insecurity Access Scale (HFIAS)			
Food secure	7	1.6	0.7 3.2
Mildly food insecure	9	2.1	1.0-3.8
Moderately food insecure	34	7.9	5.6-10.7
Severely food insecure	382	88..4	85.1-91.2

HAZ, Height for Age Zscore; *WHZ,* Weight for Age Zscore; *Se,* Selenium; *Cu*, Copper; *As,* Arsenic; *Hg,* Mercury; Pb, Lead; Cd, Cadmium; CRP, C reactive Protein.

Table 2 highlights concurrent nutrition problems among children of Popokabaka at different levels:

Stunting was common in Popokabaka, with more than half of the children (56%) affected and one-tenth (11%) exhibited wasting. Anemia was also common at 68.1%, and a high malaria prevalence at 86.8% using a falciparum rapid test. Despite the high prevalence of anemia, we found less iron deficiency (1.8% using CRP- adjusted ferritin and 12.9% using transferrin saturation).

Zn and Se deficiencies were widespread among children in Popokabaka, respectively, 64.6% (95CI 59.8–69.0) and 84.1 % (81.4–87) (see Table 2), whereas Cu deficiency was found only in 6 of 412 (1.5%) children.

The results showed that As, Hg, Cd, and Pb were detected in 95.6%, 66.0%, 19.4%, and 13.1% of the samples, respectively (Table 2). More than half of children (59.7%) had quantifiable arsenic values, while Hg, Cd, and Pb were only quantified in less than 10% of children without any toxicity level.

The food security (food consumption and food access) measured at the household level was largely inadequate: The analysis of diet quality through the FCS revealed that only 18.6% of the 432 households visited had adequate food consumption. Moreover, food consumption was poor in 40.7% of the households and borderline in another 40.7% (see Table 2). Diversity in diets was reported as being low (30.8%), meaning that two-thirds of the community did not have a diversified diet. When assessing households' access to food, the HFIAS revealed a high prevalence of food insecurity in Popokabaka: only 1.6% of households were food secure, while 88.4% were severely food insecure.

4.3. Associated Factors with Nutrition Outcomes

We search for Associated factors of nutrition outcomes in each paper I- IV. We present in Table 3 adjusted estimates of end factors identified as Associated factors of specific nutrition outcomes

Table 3 . Associated factors of nutrition outcome from the biomarker survey among children of Popokabaka in 2019

Model I. **Anemia** *(by multivariate logistic regression)*	**Adj. OR**	**CI**
▪ Fever in last two weeks	1.71	1.08 -2.70
▪ CRP > 5mg/dL	1.65	1.05 -2.59
▪ Malaria	4.08	2.18 – 9.68
Model II. **Selenium** *(by multivariate linear regression)*	**Adj. OR**	**CI**
▪ CRP > 5mg/dL	1.92	1.77 – 2.32
▪ Height-for-Age	0.32	0.11 – 0.54
Model III. **Zinc** *(by multivariate linear regression)*	**Adj. OR**	**CI**
▪ Living on the western side of the Kwango River	16.1	3.07 – 29.2
Model IV. **Meat Consumption** *(by negative binomial regression)*	**Adj. PR**	**CI**
▪ HFIAS	2.69	2.67 – 0.011
▪ Wealth score	0.24	0.10 – 0.37
Model V. **Fish Consumption** *(by negative binomial regression)*	**Adj. PR**	**CI**
▪ Livestock	0.51	0.09 0.93

With OR Odds Ratio, PR Prevalence ratio

The multivariable logistic regression analysis in Model I showed that malaria (OR, 4.08 (2.18–9.68)), fever during the previous two weeks (OR, 1.71 (1.08–2.70)), and signs of inflammation (CRP > 5 mg/L) (OR, 1.65 (1.05–2.59)) were associated with anemia.

Using the multivariable linear regression analysis, models II and III fitted Associated factors for Zn and Se levels, respectively. Regarding Zn, only the location side of Kwango River determined the serum Zn levels. Children living in villages on the western side had higher serum Zn than those from the eastern side (increased by 5.95). Selenium was positively related to height-for-age z-score, indicating that the more stunted the child was, the more Se-deficient he was. Consistent with Zn, serum Se was more concentrated in children on the western side of Popokabaka. Inflammation negatively determined the Se level, even after adjustments.

Model IV and V considered the multivariate negative binomial regression to determine count outcomes(frequency consumption of meat and fish): HFIAS negatively determined the consumption frequency of the meat and fish food groups. This means that animal micronutrient sources are related to household food security. The higher the household food insecurity, the less frequent the consumption of these micronutrient-rich foods. The wealth index score positively determined the consumption frequency of meat. This means that an animal's source of micronutrient food is related to wealth. Wealthier households had more frequent consumption of these food groups than poorer households. Having livestock as subsistence activity also showed a positive relationship with fish consumption frequency.

4.4. Spatial variability and dependency of Zn-As and Se-Hg

Based on the linear statistical association between As and Zn and Se and Hg (see Paper 3), we searched for Local bivariate spatial association to establish a geogenic relation between Zn and Se deficiency and the role of heavy metal complexation. Figure 4 shows this spatial association.

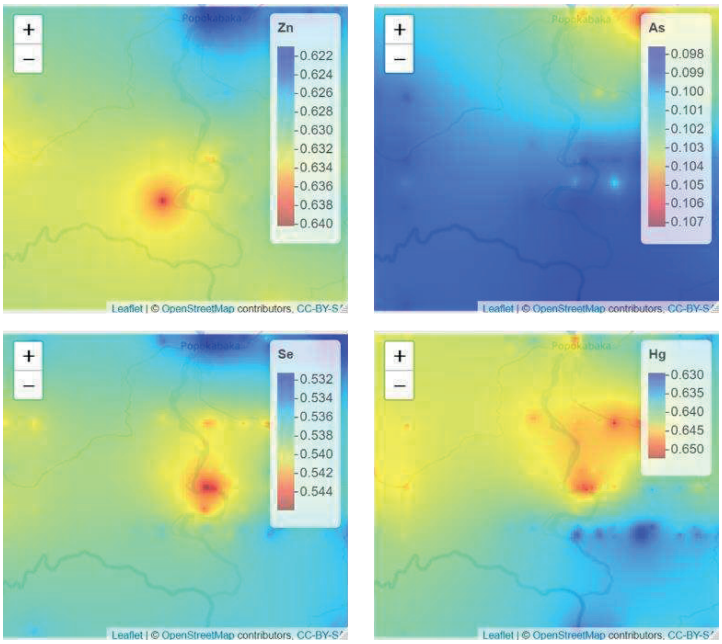

Figure 4. Spatial distribution and variation in As, Zn, Se, and Hg in Popokabaka

The spatial pattern indicates a low Zn concentration in the northern part of the study region, where As is more concentrated. In addition, the spatial distribution of Se and Hg is almost the same, with a high concentration around the Kwango River. Using the global bivariate spatial association index, the spatial distribution showed a significant spatial dependency between Hg and Se (L = 0.12, P value <0.001), implying that high concentrations of Hg were spatially associated with high concentrations of Se. A significant spatial discrepancy was observed between As and Zn (L = - 0.07, P value < 0.001). This means households with a high concentration of Se were significantly associated with high concentrations of Hg. In contrast, high values of Zn were significantly associated with low values of As.

4.5. Food Market outcomes

Table 4. Availability and average cost (per 1000 gr) of food items recorded at Popokabaka markets

	Availability 859 (100.0)	**Average cost+ CI** 95%			
		in Congolese currency		in US dollars	
Fish and sea products	46 (5.4)	76,502	(46,108 – 106,896)	38	(23 -53)
Meat	14 (1.6)	59,744	(13,132 – 106,348)	30	(7-53)
Insects	31 (3.6)	41,156	(23,573 – 58,739)	21	(12 -29)
Milk and dairy products	19 (2.2)	30,920	(15,293 – 46,555)	16	(8 -23)
Chicken/eggs	10 (1.2)	27,574	(9,480 – 45,668)	14	(5 -23)
Condiments/spices	161 (18.7)	26,444	(21438 – 31,450)	13	(11 -16)
Manufactured foods	114 (13.3)	18,915	(12941 – 24,890)	10	(6 -12)
Green leaves	102 (11.9)	12,679	(5881 – 19,477)	6	(3 -10)
Oleaginous	129 (15.0)	10,118	(7964 – 12,273)	5	(4 -6)
pulses	22 (2.6)	8,747	(5906 – 11,589)	4	(3 -6)
Fruits and other vegetables	91 (10.6)	6,919	(4,791 – 9,048)	4	(2 -5)
Cereals	38 (4.4)	5,927	(2,289 – 9,566)	3	(1 – 5)
Tubers and roots	82 (9.5)	2,775	(1,829 – 3,722)	1	(1 – 2)

Conversion rate one us dollars = 2000 Congolese currency, on 18 Oct 2022.

In total, 859 food items were recorded, listed, weighted, and evaluated for cost per 1000 g. Table 4 presents the distribution of food type availability across 13 food group categories. Green leaves, oleaginous products, condiments/spices, and manufactured foods were the most available and represented popular food groups sold at Popokabaka's markets. Milk, meat, chicken, eggs, and pulses appeared rare at Popokabaka's markets. Additionally, Figure 5 displays the most popular food item within each food group available at Popokabaka's markets.

Regardless of the types of food, the most expensive group foods were part of fish/sea products, meats, insects, chickens, and milk groups. Table 4 presents the mean food price in Congolese francs (estimated in US dollars) per 1000 g.

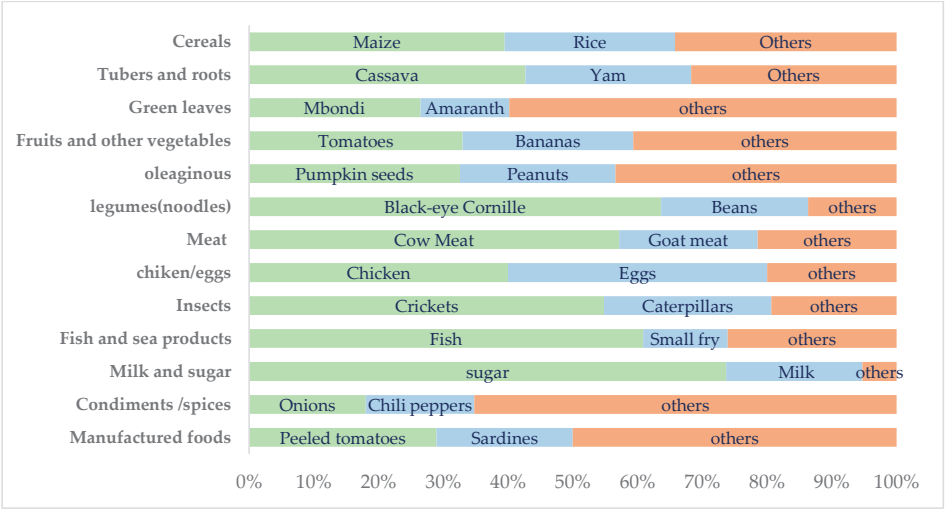

Figure 5. The most popular foods items in each food group found in Popokabaka markets

4.6. Drivers of food production in Popokabaka

Food production was assessed through a qualitative analysis of barriers and opportunities. Fifty-five individuals participated in focus group discussions and key informant interviews. Twenty-six of the participants were females. They had the following main activities: 21 farmers, eight fish farmers, 23 livestock producers, and three civil society and community leaders. Most of them identified themselves as being affiliated with community corporations. Data analysis identified key themes related to barriers and opportunities to food production in Popokabaka. Table 5 presents themes and sub-themes.

Participants discussed and explained to us the different sources of food in Popokabaka. Statements revealed that local production exists but is for household purposes. It is varied in terms of sources and may be sustainable for the community if they increase production.

> *"Here in Popokabaka, we have maize, groundnuts, cassava(fufu), and cassava leaves, which come freshly from farms, and we eat them. There are also foods from picking, as well as caterpillars and fumbwa. We also import foods from Kinshasa. We pick other foods in the neighboring villages, and we come to sell them here. Mbodis and mushrooms all come from the forest."*
>
> ***"Male participant _FGD 3_Fish farmers FGD***

Barriers included the lack of adequate infrastructure for food production, the lack of motivation in food production activity, the lack of support and control systems, and rudimentary techniques. Overall, the community of Popokabaka relies more on plant cropping activities than livestock and fishing. Despite this, they mostly show a lack of motivation to improve crop production and rely on cassava, maize, and groundnut for home consumption:

> *"We have this will and courage to work and increase our production, but the problem is that we do not get profit from it. Merchants benefit from it a lot. When you sell your products at the merchants' price, you lose a slightly high percentage, 50% of the average revenue you would gain as a retailer. We understand that the road is bad, but this fact also discourages producing in large quantities."*
>
> ***Male participant _FGD 4_APDMC Association***

Participants revealed challenges with livestock production, animal welfare, and lack of veterinarians and vaccines. In addition, it was rudimentary fishing activity, explaining the weak production of animal food sources.

> *"During the 1990s, there was the government's involvement in vaccination campaigns against livestock pests, and there were no tremendous losses compared to what we have now; however, the smallholders today are left to their own fate; they record enormous amounts of losses from their livestock. Although the dry season is known as the period in which livestock pests peak, in Popokabaka, we experience losses at any time of the year. Epidemics that were rare have become routine and permanent. All animals are concerned: pigs, goats, cows, and poultry. We suffer greatly, and this limited livestock provides an animal food source for the community. We truly need help."*
>
> ***Male participant _KII 3_Popokabaka veterinary leader***

Participants shared opportunities statements on Community acceptability, Foods locally produced, Food production activities, and Soil fertility stressing acceptance of items brought to them. They recognized that Popokabaka soil is fertile to any product implying that they will farm any nutritious imported crop with seeds, equipment, and technology provided to them. They also share a high acceptance and desire for new species and varieties introduction for crop and fish farming.

> *"Our soil accepts any crop. Because many of the crops that we use today have been imported, we have tried them, and the soil has responded well. For example, there were not any beans here; we brought them, and people tried them. Today, the soil is responding well. Apart from beans, we also brought maize; people tried it, and maize gave them a good yield. There are a few households that use onions, which we brought; the yield grew, and the production was good. This means that the soil in Popocite is very fertile for everything we have brought, even in the old day. There were a few people who grew soybeans, and the yield was good, which means that if a crop is brought to us now, the production will be good because the soil we have is wealthy for everything."*
>
> ***Female participant _FGD 3_Association'' SONGAMBANDU''***

Table 5. Drivers of food production in Popokabaka

	Themes	Sub-themes
Barriers	**1. Lack of adequate infrastructure**	Market on fixed days Lack of adequate equipment Bad road conditions Limited crop/animal species Lack of support Land ownership
	2. Lack of motivation	Domestic/insufficient production Lack of interest in production activity No gain in trading Limited affordability
	3. Pests/diseases	Lack of experts (veterinarians and agronomists) Animal pests
	4. Traditional technology	Risk of encountering crocodiles while fishing Seasonality constraints Traditional farming techniques
Opportunities	**1. Community acceptability**	Community's acceptance of new foods Existing communication channels A desire for new varieties/species
	2. Foods produced	Popular cultivated plant Locally produced foods
	3. Food production activities	Crop River fishing/fish farming Food production Picking/hunting Livestock/poultry Importation from Kinshasa
	4. Soil fertility	New plant varieties Soil environment

Chapter V. General Discussion

5.1. Discussion of the results

Iron deficiency anemia

The WHO argues that almost 50% of anemia could be caused by ID and that ID is the "single" largest contributor to the anemia burden [56]. However, the present study reported very low iron storage depletion (12.9% according to TSAT and 1.8% according to regression-adjusted ferritin biomarkers) with high anemia prevalence (68.1%) among children of Popokabaka. This is consistent with the findings of Harvey-Leeson [16], in the western part of the DRC (Kongo Central Province), and Bahazire [57], in the eastern part of the DRC (Kivu Province). Gebreegziabher et al. [58] reported the same situation among women of reproductive age in rural southern Ethiopia.

By contrast, Muriuki et al. investigated iron prevalence across five African countries [59] and found a much higher pooled prevalence of ID, 34.3%. Another reason might be the systematic iron supplementation strategy for children with anemia in Popokabaka, causing iron repletion, also possible iron supplementation (syrups or tablets) administered at home to anemic children by parents in Popokabaka. Taking into account the low consumption of animal-source foods in Popokabaka, another possible explanation is the frequent consumption of iron-rich vegetables (e.g., cassava and amaranth) [60] and the widespread self-prescription of iron tablets [57]. Based on the existing literature (23,24), we anticipate that hemoglobin genetic disorders (e.g., the sickle cell trait) and high iron supplementation may be common explanations in the malaria-endemic context. The biological pathway involves increased iron absorption capacity in some conditions, even though the storage capacity is normal.

The difference between TSAT and adjusted ferritin ID prevalence is questionable and needs clarification. As known, under the high prevalence of malaria and inflammation, the prevalence of ID according to ferritin could be underestimated because of the bias of ferritin toward higher concentrations although adjustments. Motadi et al. [61] also found a much higher prevalence according to TSAT (12%) than adjusted ferritin (2.3%). Moreover, Muriuki et al. [59] reported that TSAT is the most sensitive and specific marker in inflammatory conditions compared to the gold standard, ferritin, in the absence of inflammation. Dignass et al. [62] also suggested using TSAT rather than ferritin in the context of chronic inflammatory diseases. In a review published in 2020, Capellini et al. [63] emphasized that, in the context of inflammation, the TSAT biomarker is more sensitive and less biased than a ferritin cutoff of 100 μg/L.

In our study, fever in the previous two weeks, malaria status, and inflammation status (CRP>5 mg/L) were the stable associated factors of anemia. Although we did not assess other illnesses, such as helminthic infections [64,65] , we hypothesize that the unexplained anemia could be caused by other conditions that we did not assess in our study. For instance, Chaparro and Suchdev [66], when framing causal models for anemia Associated factors, emphasized malaria, poor sanitation, underweight, inflammation, stunting, and vitamin A deficiency.

Zinc and Selenium Deficiencies and the copper/zinc Ratio

Our results revealed that, in the context of Popokabaka, where children are exposed to a cassava diet, Zn and Se deficiencies are severely prevalent, with normal Cu levels implying a mineral imbalance (Cu/Zn ratio as 2:1) that potentially lead to cognitive development and growth impairments. This coexistence of Zn and Se deficiency and severe stunting in Popokabaka suggests grounded and permanent causal pathways that also require specific long-term and effective food-based strategies [67] intervention to reverse the situation.

Despite its common distribution in sub-Saharan region [68], Zn deficiency was found to be more profound among children of Popokabaka (Kwango Province) compared to those from Kongo central and south Kivu provinces DRC (25%–29%) [16]. Dietary zinc adequacy depends highly on the bioavailability of zinc-rich foods. This statement may explain the difference across these provinces [69]. For instance, communities in Kivu province are pastoral, commonly practice livestock, and have great accessibility to meat, beans, and potatoes that are culturally accepted as staple foods. The Kongo central province also has varied food availabilities because of its geopolitical situation as the entrance of food importation from the Atlantic Sea. Thus, dietary customs are diversified with possible high Zn intake in communities. By contrast, Popokabaka is enclaved, entirely rural, hard to reach, and almost inaccessible for food importation from big towns in DRC. Crop variety and animal-source foods are limited. It is known that food accessibility contributes to the increasing food dietary diversity and nutritional intake in a community [70]. These studies imply high variations of serum Zn deficiency across DRC provinces and disparities to consider any further national-scale survey in DRC.

Similarly, Se deficiency is established to be linked to sub-saharan food systems [71], mainly through limited access to animal sources. Dietary customs or habits may also influence the Se intake exposing poor communities to limited accessibility to the best source of Se (meat, fish, and eggs) and at higher risk of Se deficiency [72]. The cassava-based diets in Popokabaka lack Se and seleno-proteins (seleno-methionine and seleno-cysteine). Consumption of such a staple food without significant animal food sources limits the Se intake, and its tissue stock and exposes children to serious health problems: iodine disorder diseases (IDDs) [73] and konzo [32] are some examples. Although goiter and cretinism have been almost eliminated through the successful iodine salt fortification strategy, the presence of a possible iodine deficiency should be rethought. Konzo, associated with highly concentrated cyanate precursors of cassava plants, is also prevalent in the Popokabaka region 33–35,75 and should benefit from preventive research focus on selenium.

An increased Cu/Zn ratio may indicate growth impairment, cognitive abnormalities, risks of bowel diseases, and increased oxidative stress and cardiovascular disease [74,75]. We reported a Cu/Zn ratio of 2:1, which was twice the recommended standard (1:1), implying an urgent Zn strategy (Zn fortification or supplementation) to increase Zn, thereby correcting the Cu/Zn ratio among children of Popokabaka.

Arsenic, Mercury, Interaction As-Zn and Hg-Se

Arsenic was surprisingly detected in almost all children (95.6%), with more than half (59.7%) having quantifiable values. Since the arsenic crisis report from Bangladesh [76] and the recent research in Africa [77,78], Arsenic has become an increasing public health interest. It is spatially abundant and contaminates water, soil, sediment, fish, rice, and other vegetation [79]. The situation in Popokabaka is quite typical: communities drink untreated water but have relatively low consumption of fish/seafood and rice. The geographical inaccessibility of this area also limits imported sources of such foods as fish from the ocean. Arsenic exists in different forms in nature from non-toxic to carcinogenic[79]. But our study could not differentiate the total As concentration we measure. Biomonitoring and deeper exploration are needed to urgently establish environmental causal pathways that could help adapt defensive measures to prevent health and nutrition damage in communities. Linear and geospatial analysis that the arsenic was more commonly found in children with more profound Zn deficiency. This interaction between As and Zn is supported in the literature. Kader et al.[80] reported that the presence of the two minerals in the soil could lead to chelation: Zn uptake in plants is significantly reduced in As-containing soils. This geogenic source (soil) of Zn deficiency should also be explored when developing strategies to tackle Zn deficiency in Popokabaka.

Our data revealed that the 95th percentile of Pb was at 12.8 µg/L, with a detection proportion of 66% of children and quantification in 8.2%. Contrarily to arsenic, Hg is highly toxic, unnecessary for human metabolism, and may cause, at a trace level, severe damage to the nervous system, development, and behavioral performance[81]. Literature [82–87] supports that Hg contamination mainly comes from mining or industrial sources. However, Popokabaka is a rural and non-mining known region. Fish consumption from the local river came to us as most of the Hg sources in Popokabaka. Even if overall fish consumption is low there, it may likely be higher along the river than in other areas. The literature supports that a diet favoring seafood is associated with a high level of blood Hg [88]. Another argument in favor is that we found a positive association (statistical and geographic) between mercury and selenium. Both elements are common in fish, and the high detection/exposure area is along the river. This environmental source and others should be of priority interest in further exploration.

Micronutrient- rich food consumption

Rural settings should have plenty of diverse and fresh micronutrient-rich foods, and Communities living there should not have MND problems. We assess food consumption and dietary patterns in Popokabaka households in light of the biomarker level. The results reveal that Popokabaka households experience a high prevalence of severe food insecurity-access (89%) based on the HFIAS and a high prevalence of poor food consumption (40.7%) based on FCS. Diet is plant-based with limited animal-source foods consumption. Meat, fish, insects, milk-dairy products, and egg consumption were uncommon, and green leaves remain the leading micronutrient-rich food eaten. Such a picture has been described by Ecker *et al*.[89] in three rural settings (Rwanda, Uganda,

and Tanzania) in Eastern Africa, suggesting a poor quality diet in contrast with wealth agriculture opportunities. Multivariate analysis showed us that Food security, wealth index, and livestock ownership were Associated factors of micronutrient-rich frequency consumption in Popokabaka.

Food security at a glance

To better contextualize long-term action and interventions to reverse MND in Popokabaka, we comprehensively analyzed food security at different levels of food production: Household, Market, and on-farm levels. We connected both quantitative and qualitative information on food security through 3 convergence mixed design pathways: They were:

The poor diet quality pathway: linking the high level of poor food consumption in households to low affordability of animal source food at a market level, grounded by a lack of adequate animal production systems and techniques. This pathway is supported by Baltenweck et al.[90] and Hetherington et al.[91], who reported also established relationships between livestock ownership, ASF consumption, and nutritional outcomes in children within the same households in rural villages in sub-Saharan Africa.

The culture-grounded dietary pathway: linking the low dietary diversity in households to the high availability, at the marketplace, of green leafy foods and culturally grown crops plant, sustained by a lack of motivation to an improved and diversified Agriculture. Cassava, maize, and groundnuts are for Yaka communities as part of their culture. Although most of the research [30,35–37,92] conducted in the same area tried to link the traditional monotonous cassava diet to the prevalence of Konzo disease, Key informants shared more acceptability to new crops and experiments and claimed for their soil fertility.

The risk perception pathway: contrasting the food access anxiety in households (expressed by the high level of severe food insecurity in households) with the unexpected satisfaction of buyers in a context of high community acceptability and commitment to their current situation. Based on the HFIAS index construction, a considerable proportion of households are anxious and uncertain about their respective households' food supply, the insufficient quality of the food they have access to, and their insufficient food intake. People did not stock up on food at the household level; indeed, they regularly supplied themselves with food on each official market day. As they could get enough food from what was available, they felt satisfied and developed a kind of positive defiance in the face of severe food insecurity. Some authors [93–95] have described how the perceived risk of food insecurity and this high level of defiance are used to create local solutions.

5.2. Methodological consideration

- **Strengths**

The strength of this study is its rigorous methodology. We successfully conducted a biochemical study in a hard-to-reach rural settlement with many sample-conservation constraints for representative children population from Popokabaka using a probabilistic sampling scheme of a large sample size. Furthermore, we tested and developed reliable handling standard operating procedures (SOP) that limit metal contamination risks and solve cold chain challenges in such complex research conditions.

Biomarkers (ferritin, zinc, and selenium) we measured in this study are important parameters of the acute phase of inflammation response. Without adjustment for inflammation, we may report wrong and biased estimates. Our study followed the BRINDA recommendation and used the regression model to adjust these biomarkers estimates for the C Reactive Protein.

As knowledge production, our study provided community-based Information on the serum level of multiple essential TEs and heavy metals from this rural, hard-to-reach area for the first time. Unless these results are generalizable to the larger child population of Popokabaka, similar nutrient imbalances might be expected for similar rural regions in DRC. This was possible because of The lab analysis method used in this study, ICP–MS, known as the most accurate for trace elements.

To account for dietary patterns and micronutrient-rich food consumption, we built a model based on count response variables (and negative binomial regression). Using aggregated binary response variables (and logistic regression) would lead to losing valuable information, power decrease, and statistical inefficiency. This provided us with a more accurate interpretation of the Associated factors that work to increase the frequency consumption of micronutrient-rich foods.

The spatial association and dependency of Zn-As and Se-Hg we discovered in our study added value to the bioavailability of Zn and Se in Popokabaka and implied environmental actions to be taken against As and Hg together with nutrition actions. The sophisticated spatial interpolation provides the best linear unbiased estimates and highlights local.

As food insecurity emerged as a common underlying determinant of micronutrient deficiencies, we suggested mixed methods analysis as an alternative to multiple confusing specific indicators. The methods allow integration and link of Information through pathways that can lead to the development of adapted actions.

5.2.2. Limitations and validity

5.2.2.1. Internal validity (Bias and Counfounding Management)

Althouh we are confident that estimates provided here are valid internally, we discuss in this section how we managed some potential biais and confounders.

A/ Potential biases

A biais is defined as a systematic distorsion of the estimated effect of an exposure away from the truth. In this study, biais may have occurred at any phase of our research, including data collection, data analysis, interpretation and publication. We discuss below major bias

- ***Selection biais***: as described in the population and sampling section, the present study was restricted to ''technically accessible areas'' from the place (Reference Hospital) where blood was processed to serum. This was to prevent and avoid damage to proteins and possible hemolysis within samples due to long distances from the field to the referral hospital for serum separation. We can expect the distant clusters might have equal range of estimates we report here or higher prevalence taking to account their inaccesibility and limitation of food supply.

- ***Information biais***: Measuring trace elements is usually suggestive to contamination from external source, thus, equipment and procedures should be selected with caution to avoid contamination from external source. In this study, we used trace-element-free equipment and procedures were standardized. Data collectors and phlebotomists were trained to the same operating procedure. During data analysis, outcomes we reported in this study were measured using the best-todate biomarkers to avoid missclassication. Any variable transformation was based on official recognized cut offs.
  In our study, we focused on minerals. We acknowledge that the analysis of more biomarkers like vitamins would have added scientific value, but we were limited with ethics when we decided to draw a huge volume of blood, 10 mL, to have enough serum and plasma. Regarding heavy metal toxicity, it was also impossible to confirm the chronic exposure and accumulation history using blood samples, although samples were drawn under fasting status. The liver and kidneys highly and continuously regulate heavy metals' serum levels. Further studies that use matrices such as nails, hair, and skin could complement and improve our chronic risk and cumulative toxicity. Finally, most dietary indicators were based on recall memory, with an increased possibility of recall bias. To minimize this classification bias, data collectors were trained to ask the mothers to remember the exact food item they had prepared each day of the previous week before giving the frequency of each of the 16 items.

b/ Counfouding

Counfonding is an effect of a variable that causes a distorsion in the estimated effect of an exposure of interest with a given outcome because of its mixed effect with that exposure. Inflammation statut in children was the main confounding factor that we controlled in this study. Ferritin and zinc are known as positive and negative biomarkers of acute response to inflammation, respectively. Ferritine level raises up and zinc fail down during acute phase of inflammatory conditions. Our children populations show half density of inflammation (History of fever in the two preceding week, CRP > 5 mg/dL, Malaria). Without controlling these biomarker for inflammation we should have baised estimates. To do so, we have , in this study, control both biomarkers for CRP using the regression technique as recommend by BRINDA and BOND Series. In addition, we consider TSAT in preference of the adjusted ferritin. Moreover, Information about others confounders(Age, sex. clinical status, mineral supplementation,..) were collected and controlled using multivariate logistic regression, even if there should be residual confounding of unknown confounders we had controlled known confounder.

For example, the description of anemia did not consider any adjustments for altitude or ethnicity. Although we did capture geo coordinates (altitude) during data collection, Popokabaka Health Zone is a relatively flat and low-altitude region inhabited by the same Yaka population. In these conditions, the misclassification of anemia would have been minor and would not have affected our results. Regarding seasonality, we know that food availability and production, which influence food consumption, may vary. Thus, the consumption patterns reported in this study may differ between the rainy and dry seasons. Our results should be restricted and applicable only during the data collection period (the dry season). We have then assessed the worst dietary scenario for that region.

5.2.2.2. External validity (study design, sample size and chance) and generalizability

We are confident that our study is generalizable to Popokabaka Health zone Children, and recommendations may be suggestive to region s with similar contexts. However, quantitative strands of this mixed methods study are composed of cross sectional studies, which are known as low in evidence of causality. Under household and market settings, the cross sectional design was used to estimate the prevalence of outcomes and their associated factors. But as temporal relationship could not be assumed , we can not assume these reported associated factors as causal. We identified some factors as possible Associated factors of nutrition outcomes. However, the cross-sectional design we used does not guarantee or ensure temporal relationships between these factors (exposures) and outcome variables of interest cannot be ensured; thus, the causality of these deficiencies cannot be confirmed from our findings.

The sample size was calculated using anemia prevalence (58%) as the outcome giving the highest maximum variance (px(1-p)) to provide minimum sample size to englobe any other outcomes under study. We report narrow Confidence interval and only retain associated factorswith non nulle(OR=1) confidence interval from multivariate analysis.

5.3. New Knowledge and policies implication

As a novel contribution to scientific knowledge, our work has brought the following inputs.

New knowledge	Policies implication
1) Children are anemic but less iron deficient. Iron deficiency might not be the main cause of anemia in malaria-endemic areas. Malaria or other inflammatory conditions may be the leading factors.	A. Strategies to reduce anemia burden should focus on treating infectious diseases (malaria), much more than systematically supplementing anemic children with iron syrups or tablets.
2) *Se* and *Zn* deficiencies are common and severe in the region with an inadequate cassava diet.	B. Agriculture should focus on livestock, poultry, and fish farming to provide locally enough meat and seafood for population consumption.
3) *As* was detected in almost all children and in quantifiable levels in half of the children. As was negatively associated with Zn levels	C. Environmental exposure assessment is needed in Popokabaka to tackle MND successfully. Contaminated water and sea foods/fish from the river may be the focus.
4) *Hg* was detected in half of the children with fewer quantifiable levels. *Hg* was positively associated with Se levels.	
5) Food security, wealth, and livestock ownership positively determine the frequent consumption of micronutrient-rich food consumption, meat, chicken, fish, and insects.	D. Policies and interventions that empower local food producers should be encouraged.
6) Key informants reported that new crops (other green leaves and soybean) are successfully being tested and grown.	E. Agriculture experiments and the introduction of new varieties should be advocated.
7) Participants expressed the urgent need for drugs and vaccines and training in modern techniques of raising livestock and farming fish.	F. Pharmacies and vaccines and veterinarian training needs to be initiated.

5.4. Theory of change

The theory of change is a practical tool and framework for successful transformative actions. It illustrates destinations of progress, fixes targets, and the routes to achieving progress. The framework is already established and needs to be provided with adapted activities with regards to components. We suggest following activities for a visible change in the trends of MND for the five next years

- **Policies**
    1. Multiple micronutrient powders sprinkles (Health Zone, Pronanut, UNICEF, PAM)
    2. Control of infectious diseases (Health zone, SAVE THE CHILDREN)
    3. Regulation of land acquisition (Administrative stakeholders)
- **Production And Supply**
    1. Biofortification Orange Fleshed Sweet Potatoes (Harvestplus)
    2. Provisions of equipment for agriculture (FAO)
    3. Women empowering in Agriculture production (PROYAKA, ISCO, CARITAS, CONGO DORPEN)
- **Delivery**
    1. Promotion of livestock- Training on breeding (ISAV, FAO)
    2. Home gardening of micronutrient rich food (PROYAKA)
    3. Training in fish farming and husbandry (ISAV, FAO)
- **Quality**
    1. Agriculture research on experiments on new varieties/species and quality of soil (UNIKIN, FAC AGRO, ERAIFT, KSPH)
    2. Environmental research on heavy metal risk exposition (KSPH, CRENK)
    3. WASH (PRONANUT, UNICEF ,ISCO)
- **Behavior Change Communication**
    1. Mass media on dietary diversification (JICA, ENABEL, CARITAS)
    2. Promotion of micronutrient-rich foods (JICA, ENABEL, CARITAS)
    3. Suporting Radio channels communication (JICA, ENABEL, CARITAS)

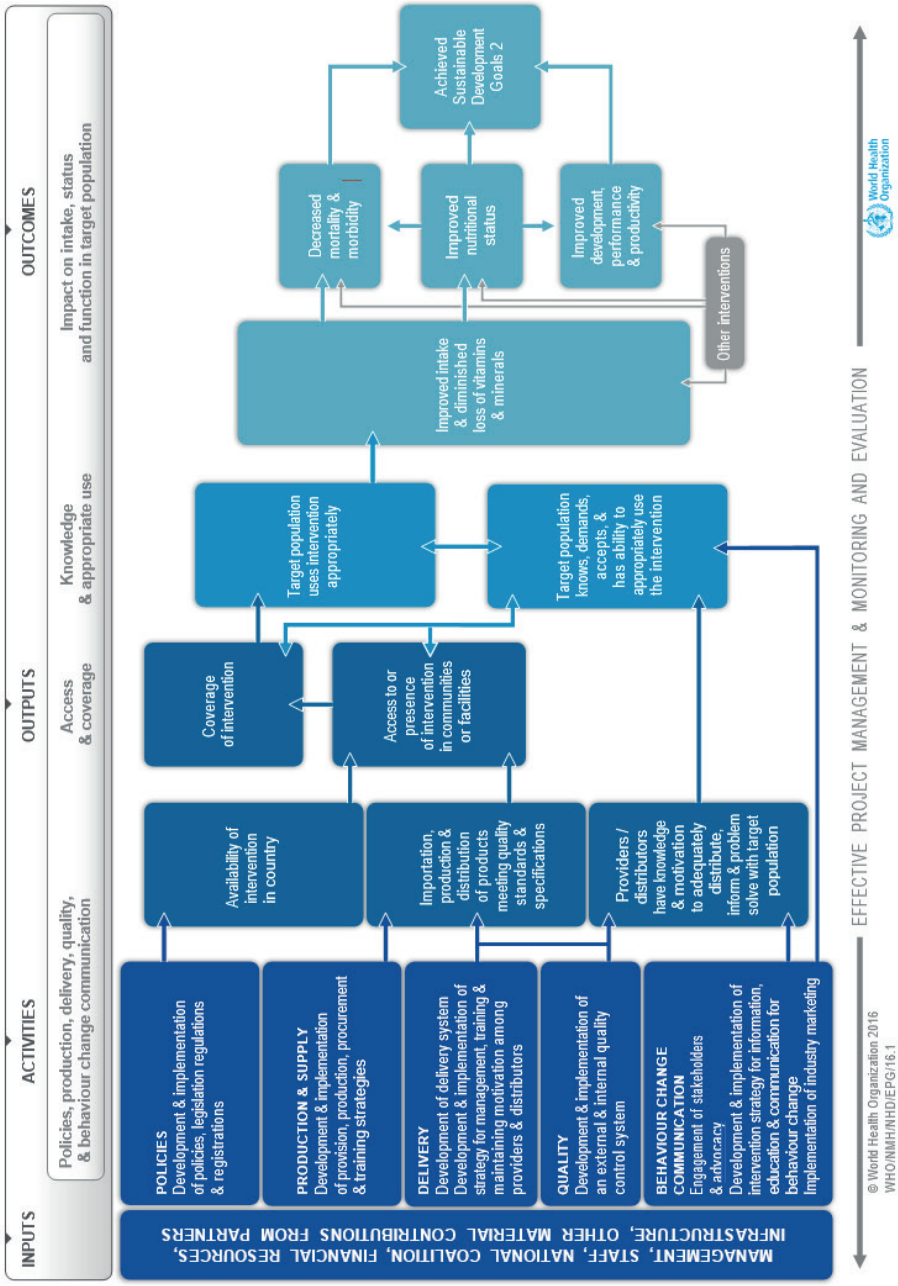

Figure 6 Adapted theory of change from WHO to reverse Micronutrient deficiencies in Popokabaka.

Conclusion and Perspectives

The present demonstrated a low iron deficiency prevalence within a context of high anemia and malaria prevalence in Popokabaka. Not Iron Deficiency, but Malaria is a major contributor to anemia in Popokabaka. We suggested that anemia-control strategies in Popokababa focus on malaria prevention and other childhood infectious-disease controls for effective impact.

We brought reliable data on serum Zn, Se, andd Cu concentration in children under five years, residing in a cassava diet area. In Popokabaka, Zn and Se Deficiency are severe, with elevated Cu/Zn ratio. Urgent cost-effective, and sustainable strategies and actions must be implemented to avoid irreversible cognitive and growth damages due to these imbalances.

The high occurrence of As and other Hg detection reported in this study implies that Popokabaka should be considered an area with certain HM hazards. Our study discussed the geogenic source (soil) and the impact of these metals in limiting the bioavailability of essential micronutrients. A deeper exploration of contaminated food and drinking water is needed to establish environmental causal pathways and adapt defensive measures to prevent toxic damage in communities.

To better approach the main underlying cause of micronutrients in Popokabaka (food insecurity), we developed a theory of change with effective and adapted activities like the development of livestock, the etablishment of an adapted communication about nutrition to change engrained dietary habits, the empowerment of the community and regulations and policies.

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Appendix

- **Ethical Clearances**
- **Published Papers**

Ethical Clearances

REPUBLIQUE DEMOCRATIQUE DU CONGO
Ministère de l'Enseignement Supérieur et Universitaire
Université de Kinshasa
ECOLE DE SANTE PUBLIQUE
COMITE D'ETHIQUE

No d'Approbation: ESP/CE/.002./2019

Kinshasa 28 janvier 2019

A Monsieur Mbunga Branly
Assistant à l'Ecole de Santé Publique
Université de Kinshasa

Objet : Approbation de votre protocole de recherche intitulé « Carences en micronutriments à Popokabaka, en RDC »

Cher Monsieur,

Le comité d'éthique de l'Ecole de Santé Publique de l'Université de Kinshasa a examiné le protocole de recherche (et ses annexes) dont l'intitulé est repris en marge.

Le projet ayant été jugé pertinent et vu que les principes d'éthiques seront bien pris en compte lors de la mise en œuvre du projet, le comité émet l'avis favorable et autorise l'exécution du projet pour la période allant du 29 Janvier 2019 au 28 Janvier 2020.

Tout nouvel amendement au présent protocole devra au préalable être soumis au comité d'éthique avant de devenir effectif. Tous incident devra être rapporté au comité d'éthique et devra faire l'objet d'un rapport écrit.

Veuillez agréer, Monsieur, l'expression de nos salutations distinguées.

ESP UNIKIN
Scientia Splendet et Conscientia
COMITE D'ETHIQUE

Prof. Dr Patrick Kayembe Kalambayi
Président du Comité d'Ethique

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK vest	Jessica Svärd	55978497	30.11.2018	2018/1420/REK vest
			Deres dato:	Deres referanse:
			05.11.2018	

Vår referanse må oppgis ved alle henvendelser

Ingunn Marie Stadskleiv Engebretsen
University of Bergen

2018/1420 Micronutrient deficiencies in PopoKabaka, DRC

Institution responsible for the research: University of Bergen
Project manager: Ingunn Marie Stadskleiv Engebretsen

With reference to your application regarding the abovementioned project and the respone submitted 05.11.2018. The Regional Committee for Medical and Health Research Ethics (REC Western Norway) reviewed the application in the meeting 15.08.2018, pursuant to The Health Research Act § 10. The response was reviewed by the committee chairman.

Review

The Regional Committee for Medical and Health Research Ethics (REC Western Norway) reviewed the application in the meeting 15.08.2018.

REC Western Norway asked for a response to the following questions from the committee:

- Describe the planned procedures for follow-up. Will an intervention be initiated? Will participants be informed and referred to further follow-up?
- A revised information sheet must be sent to REK vest.

Response from the project manager
The researcher team thanks the committee for their thorough feedback and this led to an indepth discussion in our researcher team on how we should deal with the problems raised in a scientifically sound and ethical way. We hope you find our answers in agreement with your concerns.

1. Is there any adequate procedure if serious vitamin or iron deficiency is detected?
Describe the planned procedures for follow-up. will an intervention be initiated?

In the context of the present study, no single mineral or vitamin should be given as a drug or supplement to any respective child found deficient. The reason is that to date there are no guidelines, no procedure nor cut-offs for individual micronutrient deficiency treatment. For example, if a child is found selenium deficient, we cannot provide selenium tablets to him because there is no international evidence-based guideline for such a supplementary procedure. At national level either, in the DR Congo, there is no such health policy. Another reason is that

there is risk of falling in toxicity as cut offs are unknown. These are trace elements that the body absorbs and needs at small amounts from the eaten foods.
This is a cross sectional study aiming at measuring the level minerals and vitamins deficiencies at the community level. At this stage no intervention will be initiated, but since we will conduct this study in Popokabaka, an area where local diet has been shown not to be diversified and not providing sufficient quantities of micronutrients to meet recommended intakes, participants will be provided regular sprinkles (multiple micronutrients powders): Sixty single-dose sachets for each child, consumed over 60 days. No more than one sachet a day should be used for a child. The advantage is that this strategy exists as national policy and is promoted by UNICEF even though the provision for Popokabaka area stopped years ago. Mothers should easily add/ spread to the food of the child that is ready to be consumed to meet the daily recommended nutrient intake and community workers may be used to assist mothers and follow up.
In addition, as micronutrient deficiencies often exit alongside with acute malnutrition, malnourished children will be referred to the Popokabaka hospital, where ready-to-use therapeutic foods (Pumply-nut) and ready-to-use supplementary foods (Pumply-sup) will be provided. Those pastes contain also minerals and vitamins that could help. The advantage is the well-known "Management of acute malnutrition" is practiced over the country.
In addition, Hemoglobin and malaria screening is done on spot and any severe anemia (WHOcriteria) or positive malaria-infection will be referred to the hospital.

According to the findings, we will rely on community awareness and education on nutrient rich foods instead of giving individual feedback as lab analysis results will only be available later. We will develop a communication plan of sensitization of selected nutrients that will be found more deficient in Popokabaka.

2. Will participants be informed and referred to further follow up? Do researchers have a duty to follow up?

The assessment involves anthropometry, malaria and anemia assessment on spot. Hemoglobin and malaria testing are rapid test, results for these tests will be directly given to the mother. She will be informed of that and will be referred for care at the health facilities if the child has malaria or severe anemia or moderate and severe undernutrition. The project will supply these health facilities with iron supplementation and anti-malaria medication in cases of stock-out. However, minerals and vitamins will be tested in laboratory in Bergen, so there will be a delay between the time of data collection and the time of lab analyses. We will not individually give feedback after such a period for a past point biochemical status. The reason for this is that we find it unethical and provocative to give individual feedback on results that do not have any treatment. Instead, the community level snapshot that we will get from these lab analyses will orient our community message and further intervention. But If any participant (parent) specifically requests the information from the research team, he will be able to receive feedback on the individual lab testing of his child.
Any child with visible clinical signs of micronutrient deficiency will be referred for nutrition counselling or care at Popokabaka Hospital.

3. A revised information sheet must be sent to REK vest.

A revised information sheet and a revised research protocol is attached to the respone.

The committee chairman reviewed to response.

Review

The project manager informs that each child will be provided with sixty single-dose sachets multiple micronutrients powders to be consumed over 60 days. No more than one sachet a day should be used for a child.

The project manager further informs that, as micronutrient deficiencies often exit alongside with acute malnutrition, malnourished children will be referred to the Popokabaka hospital, where ready-to-use therapeutic foods (Pumply-nut) and ready-to-use supplementary foods (Pumply-sup) will be provided.

REC western Norway finds the response from the project manager satisfactory and approves the project.

Decision:
REC western Norway approves the project.

Further Information
The approval is valid until 01.12.2020. A final report must be sent no later than 01.06.2021. The approval is based on the grounds that the project is implemented as described in the application and the protocol, as well as the guidelines stated in the Health Research Act. If amendments need to be made to the study, the project manager is required to submit these amendments for approval by REC via the amendment form.

The decision of the committee may be appealed to the National Committee for Research Ethics in Norway. The appeal should be sent to the Regional Committee for Research Ethics in Norway, West. The deadline for appeals is three weeks from the date on which you receive this letter.

Sincerely yours

Marit Grønning
dr.med. professor
Chairman

Jessica Svärd
committee secretary

Kopi til: postmottak@uib.no

Paper I

nutrients

MDPI

Article

Prevalence of Anemia, Iron-Deficiency Anemia, and Associated Factors among Children Aged 1–5 Years in the Rural, Malaria-Endemic Setting of Popokabaka, Democratic Republic of Congo: A Cross-Sectional Study

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Citation: Mbunga, B.K.; Mapatano, M.A.; Strand, T.A.; Gjengedal, E.L.F.; Akilimali, P.Z.; Engebretsen, I.M.S. Prevalence of Anemia, Iron-Deficiency Anemia, and Associated Factors among Children Aged 1–5 Years in the Rural, Malaria-Endemic Setting of Popokabaka, Democratic Republic of Congo: A Cross-Sectional Study. *Nutrients* **2021**, *13*, 1010. https://doi.org/10.3390/nu13031010

Academic Editors: Elad Tako

Received: 14 February 2021
Accepted: 18 March 2021
Published: 21 March 2021

Abstract: Iron deficiency (ID), the leading cause of anemia and the most common nutritional deficiency globally, is not well reported among children in malaria-endemic settings, and little is known about its contribution to anemia in these settings. We aimed to assess the prevalence of anemia, the role of ID using multiple parameters, and the factors associated with anemia in a malaria-endemic rural area. We conducted a community-based cross-sectional study of 432 children aged 1–5 years from the Popokabaka Health Zone, Democratic Republic of Congo. Sociodemographic characteristics, medical history, anthropometric parameters, and biochemical parameters were considered. Hemoglobin and malaria prevalence were assessed using rapid finger-prick capillary blood testing in the field. Venous blood samples were analyzed for serum ferritin, serum iron, total iron-binding capacity, and C-reactive protein (CRP) in a laboratory. Anemia was found in 294 out of 432 (68%) patients. Malaria was found in 375 out of 432 (87%), and ID in 1.8% according to diagnosis by adjusted ferritin only and in 12.9% according to transferrin saturation. ID indicators were not significantly correlated with low hemoglobin levels. Malaria, fever, and CRP > 5 mg/L were major factors associated with anemia in Popokabaka. Anemia control should focus on treating inflammatory conditions and infectious diseases among children in such settings.

Keywords: anemia; iron deficiency; children; malaria; Popokabaka

1. Introduction

Anemia, which is characterized by a hemoglobin level below 11.0 g/dL, continues to be a serious global public health problem that particularly affects young children and pregnant women [1]. The World Health Organization (WHO) reported that an estimated 42% of children aged < 5 years are anemic worldwide; the burden is even higher in Africa, reaching 62.3% [2]. According to the most recent Demographic and Health Survey (DHS) and the Multiple Indicators Cluster Survey (MICS), the prevalence of anemia among children aged < 5 years in the Democratic Republic of Congo (DRC) is around 63% [3,4].

While multiple factors are known to cause anemia, the literature [5–8] shows that iron deficiency (ID), wherein a person has low storage of iron, is the most common nutritional cause [7–9]. Over several decades, nutritional interventions have been carried out in most developing countries [7], and nationwide in the DRC [3], to strengthen the prevention of anemia in childhood. One might have expected a decrease in the incidence of anemia, but its prevalence remains high. This is particularly evident in malaria-endemic areas [10].

There is little information to date on ID and its contribution to anemia in the DRC. Large-scale surveys such as the DHS or MICS have not assessed iron parameters, perhaps because of the complex logistics and laboratory analysis required. However, some studies have been conducted, such as the study by Harvey-Leeson et al. in 2016 [11], which reported a discrepancy in ID prevalence (1% when using adjusted ferritin and 58% with serum transferrin) among children aged 2–5 years old in the Kongo Central Province, DRC. The study found that ID anemia (IDA) never exceeded 20%, regardless of the iron biomarker used. Bahizire et al. [12] conducted a study in the South Kivu Province, DRC, and reported an ID prevalence of 10.4% (using inflammation-adjusted ferritin), observing that ID contributed to less than a fifth of anemia cases in children aged < 5 years.

However, according to the WHO [13], half of the anemia cases are expected to be explained by ID. In this study, we aimed to assess the prevalence of anemia and the role of ID among children aged < 5 years who presented with anemia, to suggest specific directions for useful anemia-control strategies. We also assessed possible risk and protective factors associated with anemia.

2. Materials and Methods

2.1. Study Design and Setting

We conducted a community-based cross-sectional study in the Popokabaka Health Zone, DRC, between May and June 2019. This region is an entirely rural area located in Kwango Province, where the population is facing food insecurity and poverty [3]. The health system is organized around the referral hospital and health centers, coordinated by the Health Zone management. National strategies regarding malaria and anemia control are also implemented. For instance, iron and folic acid supplementation to women during pregnancy and Infant and Young Child Feeding (IYCF) support for children aged up to 2 years are organized at the facility and community levels [3,4]. Malaria-control strategies are integrated with other disease-control programs under Health Zone management [4]. These include community screening and treatment for mild malaria by community health workers, the integrated management of severe cases at health facilities, epidemic management, selective vector control, periodical distribution, and campaigning for the use of insecticide-treated bed nets. *Plasmodium falciparum* is the most prevalent malaria-causing species found in DRC [4]. Available information for the Kwango province shows that stunting (43%) and wasting (8%) are prevalent in children [4]. The diversity of food is limited, and the soil is sandy and arid, not permitting the growth of a wide variety of crops. Farmers preferably grow bitter cassava as a staple food because of its resistance to drought and the energy value of its roots. Animal food sources, dairy products, and sea products are seldom consumed.

2.2. Participants and Sampling

A total of 432 children aged 12–59 months were included in this study, based on a proportional sample size calculation for anemia prevalence = 0.59, a precision d of 0.075, a design effect of two, and an expected response rate of 0.80. Children were selected using a three-stage cluster sampling technique. First, we selected five clusters (health areas) among the nine accessible areas through a probability-proportion-to-size technique. The term "accessible" considered the time constraints for collecting venous blood samples from the field and being able to process them within 3 h at the Popokabaka Hospital. Only 9 of the 25 health areas fitted this technical constraint. Second, we randomly selected 3 villages in each cluster. In the last stage, an equal number of 30 households having children aged 12–59 months old were systematically selected from a detailed list pre-established by community workers in each village. Only one child from each household was selected and assessed. A parent had to provide written consent for their child to participate in the study. Children for whom the parents refused blood drawing or children who were hospitalized for any diseases in the two previous weeks were excluded.

2.3. Data Collection and Procedures

Data were collected using a questionnaire completed on tablet computers using the Survey CTO collect application. The questionnaire consisted of eight modules: household characteristics; water, hygiene, and sanitation (WASH); household food security (Household Food Insecurity Access Scale-HFIAS); child health history; IYCF; anthropometric measures; dietary practices (24 h recall and food frequency); and biochemical sampling.

Data collection took two consecutive days in each cluster, three teams working in parallel for each of the three selected villages of the cluster. A team comprising three data collectors and two phlebotomists was formed. On the first day, the data collectors visited the households, obtained consent forms, and completed the surveys for each child selected in the village. The biological mother or caretaker responded to the questionnaire while anthropometric measurements were taken for the child. When finished, the personnel provided a card to the mother with the correct personal identifier for blood collection the next day. On the second day, the phlebotomists went to the same villages at a specific identified location (the Health Center or another appropriate place) from 7 to 9 am for blood collection and hemoglobin and malaria rapid testing. The mothers were then invited to bring the children and the cards for blood collection.

The data collectors were trained by the investigator on the survey questionnaire and interview techniques, and the phlebotomists were trained by a lab expert on appropriate standard operating procedures (SOPs) and blood sample management.

2.4. Blood Processing and Management

The phlebotomist first performed a capillary finger-prick test for hemoglobin (Hgb) assessment (Hemocue 301) and a rapid test for malaria in the field. The information was recorded using tablet computers and the Survey CTO application. Additionally, the phlebotomist collected up to 6 mL of venous blood from the child using trace-element-free serum BD vacutainers (BD-368380) and powder-free sterile disposable gloves. Tourniquet application took a maximum of one minute. The collected blood was allowed to clot for at least 30 min in the field at room temperature and was then transported to the Popokabaka Hospital within 3 h. There, it was centrifuged, and the supernatant was separated at 2300 rpm for 10 min using a Hettich Rotor 32A centrifuge (Tuttlingen, Germany). The serum was aliquoted into two polypropylene vials: 0.5 mL tricoded FluidX, Brooks Life Science vials and 2 mL Sarstedt vials.

All the vials were immediately stored in a −40 °C freezer that worked nonstop on solar energy during the day and on a generator overnight in Popokabaka. When the survey was complete, the samples were transported from Popokabaka to Kinshasa (a 12 h vehicle trip with self-transported fuel) stored in liquid nitrogen. Then, every sample was stored at −80 °C in an ultra-low freezer at the Kinshasa School of Public Health for a week before being shipped on dry ice to Norway.

All the 0.5 mL vials were sent to Haukeland University Hospital (Bergen, Norway) for the analysis of serum ferritin (using electrochemiluminescence immunoassay (ECLIA)), C-reactive protein (S-CRP) (using the immunoturbimetry method), and total iron-binding capacity (S-TIBC, using the Berekna equation calculation: s-TIBC = s-Transferrin * 25.1). Two-milliliter vials were sent to the Norwegian University of Life Sciences (Ås, Norway) for the analysis of serum iron and other minerals (using the Agilent 8900 Triple Quadrupole inductively coupled plasma mass spectrometer (ICP-MS)).

Transferrin saturation (TSAT), expressed as a percentage, was then calculated as the value of serum iron divided by the TIBC.

2.5. Transformation of Variables

Anthropometric indices including the weight for height, height for age, weight for age, and mid-upper-arm circumference for age and their Z-scores were calculated using the WHO Anthro software. Wasting was defined as a weight-for-height Z-score (WHZ) < −2, stunting was defined as a height-for-age Z-score < −2, and underweight was defined as

a weight-for-age Z-score < −2. Wealth index quintiles were generated using principal component analysis on ownership variables in the household. The food consumption score was calculated using data from the Food Frequency Questionnaire (FFQ) and categorized as adequate, borderline, and inadequate consumption. The dietary diversity variable was generated from 24 h food recall, capturing the intake of common food items in the area. The Household Food Insecurity Access Score (HFIAS) and its categories were also calculated.

2.6. Anemia and ID Definition

Anemia was defined as Hgb levels < 11 g/dL [14]. According to the level, it was classified as mild, moderate, or severe when the Hgb concentrations were 10–10.9, 7.0–9.9, or <7.0 g/dL, respectively. ID was defined as serum ferritin concentrations < 12 μg/L in the absence of inflammation [14]. To account for inflammation [8], we used the regression-correction approach developed by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) [15] to estimate the prevalence of ID. The regression-correction approach followed a three-step process. First, we defined internal reference values for inflammatory markers (CRPref) as the tenth percentile [15]. Then, we estimated the regression coefficient (β) for the association between CRP and ferritin using univariable linear regression models, with ferritin as the dependent variable. Finally, we calculated CRP-adjusted ferritin values using the following equation:

$$Adjusted\ ferritin = unadjusted\ ferritin - \beta\ (CRPobs - CRPref).$$

We also estimated ID by calculating TSAT by dividing the serum iron (Fe^{3+}) levels by the TIBC. A child is said to have ID when TSAT is <20% [14].

A child was said to have malaria when there was a positive result in the rapid malaria test for *Plasmodium falciparum*.

2.7. Statistical Analysis

All the data were merged and analyzed using STATA 14.0. We performed a descriptive analysis. The median and 25th–75th percentiles are reported for continuous variables that were not normally distributed, while the mean (SD) is used for continuous variables that were normally distributed. Frequencies are used to describe categorical data. The confidence intervals (CIs) for prevalence were calculated using a normal Z-test. Moreover, an independent-sample t-test or the Wilcoxon rank-sum test were used to compare, respectively, mean or median values. A Pearson chi-squared test was used to test for association, with anemia as the outcome. The Spearman correlation coefficients were used to describe the relationships between the iron biomarkers and hemoglobin concentrations. Linear regression was used to adjust ferritin for inflammation (CRP). Bivariable and multiple logistic regression were used to identify factors associated with anemia, and the crude and adjusted odds ratios (ORs) were reported. The regression model was constructed by a forward stepwise selection approach with covariate inclusion probability (*p*-value of crude OR) < 0.20. Multicollinearity was checked for all the covariates included in the regression model. All the statistical analyses were performed at the 0.05 level of confidence.

3. Results

3.1. Characteristics of the Study Population

In total, 432 children aged 1–5 years old were included in this study. The general characteristics of the enrolled children are shown in Table A1. The boy/girl ratio was 1:1, and their median (P25–P75) age was 32 (22–43) months. Children aged 48–59 months were underrepresented compared to those in the other age groups. Fever onset during the 2 weeks preceding the study visits was common among the children in Popokabaka and more so among anemic children (63.3%) than those without anemia (45.7%) (Table A2). The results also support the existence of childhood health and anemia-control strategies implemented in Popokabaka, but not with optimal coverage (Table A2). Almost seven out of ten children had benefitted from deworming in the previous 6 months, while half

had received vitamin A in the previous six months. About one third (35.6%) were treated with iron supplements or syrups, suggesting that nutritional therapy for anemia was also practiced.

Stunting was the most common form of malnutrition, with more than half of the children (56%) affected, and every tenth (11%) child exhibited wasting. Only one in three had a diversified diet, and approximately 3% of the children were defined as being obese.

3.2. Prevalence and Distribution of Anemia and Iron Deficiency

The prevalence of malaria in Popokabaka was 86.8% (95% CI, 83.6–90.0%) according to *Plasmodium falciparum* rapid testing. We found that anemia was also common, at 68.1% (95% CI, 64.0–72.0%), with severe anemia seen among 3.3% (95% CI, 1.9–5.4%) of the children.

Half of the children (49.4%) had signs of inflammation, with CRP > 5 mg/L. This proportion was significantly higher among anemic children than nonanemic children (53.5% vs. 40.7%, p value = 0.001).

However, ID was less prevalent when taking all the iron biomarkers into account. First, considering that ferritin increases in the acute phase of inflammation, we determined the unadjusted ferritin prevalence by excluding the ferritin values of children with CRP > 5 mg/L during calculation. Only one out of 193 children presented with ID (ferritin < 12 µg/L). In another approach, accounting for inflammation as proposed by the BRINDA project [15], we found an ID prevalence of 1.8% (95% CI, 0.5–3.0%) using the ferritin biomarker adjusted for CRP. The latter approach, where the adjustment was performed using linear regression, allowed the inclusion of all the observations where we had measured serum CRP and ferritin concentrations. This prevalence was lower than those based on other indicators. Table A3 shows that the ID prevalence according to the TSAT biomarker was the highest, at 12.9% (95% CI, 9.6–16.1%).

We estimated the IDA prevalence and the contribution of ID to anemia, and the former 7.5% according to transferrin saturation and 1.5% according to adjusted ferritin. Thus, the remaining anemia cases were not iron-deficiency-related.

3.3. Correlation with Iron Biomarkers

Hgb was not correlated with transferrin saturation (TSAT) but showed a weak, negative correlation with ferritin (r = −0.17) and serum iron (r = 0.07) (Table A4). In addition, Hgb was negatively correlated with CRP, suggesting a possible role of inflammatory conditions or diseases in anemia genesis.

3.4. Association with Anemia

Table A5 shows the multivariable regression analysis with determinants for anemia among children aged under five years in Popokabaka. We found that malaria (OR, 4.08 (2.18–9.68)), fever during the previous two weeks (OR, 1.71 (1.08–2.70)), and signs of inflammation (CRP > 5 mg/L) (OR, 1.65 (1.05–2.59)) were associated with anemia. However, ID according to TSAT was inversely associated with anemia (OR, 0.50 (0.27–0.97)), meaning that the presence of ID did not imply or protect against anemia for the data collected.

4. Discussion

In the present study, we demonstrated the burden of ID and anemia using multiple biomarkers for a representative community-based sample of children in a poor part of the DRC, which, to the best of our knowledge, had not been assessed before. Our study found that anemia was highly prevalent (68.1%) among children aged < 5 years, while ID was remarkably low (12.9% according to TSAT, 7.9% according to TIBC, and 1.8% according to regression-adjusted ferritin biomarkers). This anemia prevalence seemed much higher than the previously reported national prevalence (59.9%, n = 8280) according to the latest Demographic and Health Survey in 2014 [3]. This may indicate that children in Popokabaka are at the highest risk of anemia complications in the DR Congo. Harvey-Leeson et al. [11] reported an anemia prevalence rate of 44% in Kongo Central Province, while Bahizire [12]

reported a prevalence of 46.6% in rural Kivu, both of which are areas known for long periods of civil unrest. This difference could be explained by the fact that our study was specifically carried out in a small geographical area, a "health zone", where the problem is indeed serious. In this case, the prevalence may be higher than that in a much larger research area. Another reason of this difference is that we did not adjust the Hgb levels for altitude and ethnicity, and this may have increased the false-negative proportion in our anemia classification. In other words, a reduction in this prevalence could be expected if those factors were also considered.

The WHO reports that almost 50% of anemia could be caused by ID and argues that ID is the "single" largest contributor to the anemia burden [16]. However, this was not the case in our study population. We found a very low prevalence of ID, with the highest prevalence being 12.9% according to TSAT. This is consistent with the findings of Harvey-Leeson [11], in the western part of the DRC (Kongo Central Province), and Bahazire [12], in the eastern part of the DRC (Kivu Province). Gebreegziabher et al. [17] reported the same situation in rural southern Ethiopia among women of reproductive age. By contrast, Muriuki et al. investigated iron prevalence across five countries in Africa [18] and found a much higher pooled prevalence of ID, 34.3%. The difference might be due to the high prevalence of malaria and inflammation in our population. In fact, under these conditions, the prevalence of ID according to ferritin could be underestimated because of the bias of ferritin toward higher concentrations. Another reason might be the systematic iron supplementation strategy for children with anemia in Popokabaka, causing iron repletion. Motadi et al. [19] also found a much higher prevalence according to TSAT (12%) than adjusted ferritin (2.3%). Moreover, Muriuki et al. [18] reported that TSAT is the most sensitive and specific marker compared to the gold standard, which is regression with adjusted ferritin in African children. Dignass et al. [20] also suggested the use of TSAT rather than ferritin in the context of chronic inflammatory diseases. In a review published in 2020, Capellini et al. [21] emphasized that, in the context of inflammation, the TSAT biomarker is more sensitive and less biased than a ferritin cutoff of 100 μg/L.

The high anemia prevalence with very low iron storage depletion in this highly prevalent malaria context cannot be explained in the present study. However, we anticipate, based on the existing literature [22,23], that in the malaria-endemic context, hemoglobin genetic disorders (e.g., the sickle cell trait) and high iron supplementation may be common explanations. The biological pathway involves an increase in iron absorption capacity in some of these conditions even though the storage capacity is normal. Taking into account the low consumption of animal-source foods in Popokabaka, a possible explanation is a frequent consumption of iron-rich vegetables (e.g., cassava and amaranth) [24] and the widespread self-prescription of iron tablets [12].

Another explanation could be possible iron supplementation (syrups or tablets) administered at home to anemic children by parents in Popokabaka, even though only 35% of those who took part in our study reported that their children had received iron supplementation in the previous three months (Table A1).

In a recent review, Camaschella et al. [25] explained the endocrine regulatory role of hepcidin in iron balance. According to their review, proinflammatory cytokines such as interleukin-6 increase hepcidin levels during acute and chronic inflammation, which leads to iron-restricted erythropoiesis and anemia of inflammation. According to this pathway, we would have expected a high prevalence of iron deficiency in children living in a malaria-endemic setting. However, this was not the case in our study. We found a low prevalence of ID in the children of Popokabaka. Camaschella et al. discussed another pathway through which unexpectedly elevated levels of serum iron might be induced along with anemia, especially in individuals with iron-loading anemias or chronic anemia: low levels of, the inhibition of, or the loss of control of hepcidin. We did not assess hepatic hepcidin in our study but suspect that this protein would be low and probably impacted by recurrent protein malnutrition (in our study, we found that one in ten children had acute malnutrition while one in two was chronically malnourished) or other hepcidin disorders.

We estimated the association between anemia and other factors in a multivariate logistic regression analysis, and we found that fever in the previous two weeks, malaria status, and inflammation status (CRP > 5 mg/L) were factors associated with anemia. Taking into account the weak correlation of hemoglobin with iron biomarkers, we hypothesized from these results that the remaining cases of anemia could be explained or have been caused by malaria or other diseases that we did not explore in our study. Unfortunately, we did not assess other illnesses such as helminthic infections [26,27]. Chaparro and Suchdev [28], when framing causal models for anemia determinants, emphasized malaria, poor sanitation, underweight, inflammation, stunting and vitamin A deficiency.

We used rapid malaria tests to determine the prevalence of parasitemia based only on the presence of *Plasmodium falciparum*. This could have underestimated the true prevalence of malaria in terms of parasite count. In a review of how malaria was associated with anemia, White [10] stated that, in endemic malaria contexts, young children had repeated symptomatic infections, with high rates of asymptomatic parasitemia, and often had chronic anemia.

In our study, anemia prevalence was indifferently distributed within nutritional parameters—food insecurity, dietary diversity, and anthropometric indicators—which did not explain any of the variation in anemia. This finding is in contrast to the summary findings reported by the WHO [7], indicating that multiple nutrient deficiencies of both minerals and vitamins are causes of anemia.

The present study had several strengths. This was a community-based biochemical study for a hard-to-reach rural settlement with many sample-conservation constraints. We obtained a representative study population from Popokabaka through a probabilistic sampling scheme with a large sample size. We demonstrated that iron deficiency might not be the main cause of anemia in such malaria-endemic areas, that malaria itself or other inflammatory conditions may be leading factors. We examined multivariate regression models and checked for multicollinearity to validate the associated factors found.

However, the study had several limitations. The study population was restricted to technically accessible areas in the study area, as explained in Section 2.2, to avoid damage to proteins and possible hemolysis due to long distances from the field to the referral hospital. Future research in such hard-to-reach areas could consider on-site blood processing and serum conservation for a higher representation of distant areas. We acknowledge that the analysis of more biomarkers would have had scientific value, such as soluble transferrin receptors and hepcidin. We conducted a cross-sectional study, so we cannot argue any causality for the relationships reported in this study. We think that the protective effect of iron deficiency on anemia was related to the medium sample size, to such an extent that this would become null with a bigger sample.

Despite the fact that the interpretation of Hgb concentrations requires some adjustments (for altitude and ethnicity) as recommended by the literature [29,30], for this study, we did not consider any adjustments for altitude or ethnicity. The reasons were that we did not capture geo coordinates (altitude) during data collection and that the Popokabaka Health Zone is a small, relatively flat and low-altitude region inhabited by the same Yaka population. In these conditions, the misclassification of anemia would have been minor and would not have affected our findings.

Finally, anemia has complex pathways, and there are likely to be other important factors involved that were not measured.

5. Conclusions

Iron deficiency should be considered prevalent in populations when its prevalence is more than 20%. In this study, we demonstrated a low iron deficiency prevalence within a context of high anemia and malaria prevalence in Popokabaka. ID was not a risk factor for anemia in this study. However, malaria was a major contributor to anemia. Further research with more hematological biomarkers is needed to characterize this anemia, which was not explained by ID. Due to the findings from this study, we suggest that anemia control

strategies in Popokababa focus on malaria prevention and other childhood infectious-disease controls for effective impact.

Author Contributions: Conceptualization, B.K.M., M.A.M., T.A.S., and I.M.S.E.; standard operating procedure development, B.K.M., E.L.F.G., and T.A.S.; fieldwork supervision, B.K.M., M.A.M., T.A.S., and I.M.S.E.; data analysis, B.K.M., P.Z.A., T.A.S., and I.M.S.E.; writing—original draft preparation, B.K.M.; writing—review and editing, B.K.M., M.A.M., P.Z.A., E.L.F.G., T.A.S., and I.M.S.E.; project administration, M.A.M. and I.M.S.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Norwegian Agency for Development (NORAD) and cooperation, the Norwegian Higher Education (NORHED) growing partnership for higher education and research in nutrition (GrowNut Project: QZA-0484, COG-13/0002) in the DRC, and a collaboration between the University of Kinshasa, University of Kwazulu-Natal, and University of Bergen.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Norwegian Institutional Review Board REK Committee (ref: 2018/1420/REK vest; date: 30 November 2018) and the Kinshasa School of Public Health ethical committee (ref: ESP/CE/2019; date: 28 January 2019) in Bergen. Other authorizations were requested from both the local administrative and health authorities.

Informed Consent Statement: Written informed consent was obtained from the mothers or caretakers of the children in this study.

Data Availability Statement: The dataset of this study can be made available on reasonable request to B.K.M.

Acknowledgments: The authors thank the participants and all the community members of Popokabaka for collaboration and participation in this study.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. General characteristics and anthropometry of children aged 1–5 years in Popokabaka.

Report Unit	Total	Anemia		*p*-Value
		Yes	No	
Age (months) Median (P25–P75)	32 (22–43)	31 (22–42)	33 (23–45)	0.869
Age Groups (months) *n* (%)				
12–23	124 (28.7)	86 (29.3)	38 (27.5)	0.985
24–35	120 (27.8)	81 (27.5)	39 (28.3)	
36–47	116 (26.8)	78 (26.5)	38 (27.5)	
48–59	72 (16.7)	49 (16.7)	23 (16.7)	
Gender *n* (%)				
Boys	224 (51.8)	152 (51.7)	72 (52.2)	0.927
Girls	208 (48.2)	142 (48.3)	66 (47.8)	
Height-for-Age Z-score Mean (SD)	−2.2 (1.7)	−2.2 (1.7)	−2.0 (1.7)	0.255
Weight-for-Height Z-score Mean (SD)	−0.6 (1.3)	−0.7 (1.2)	−0.3 (1.4)	0.011
Weight-for-Age Z-score Mean (SD)	−1.5 (1.4)	−1.7 (1.3)	−1.3 (1.3)	0.014

Table A1. *Cont.*

Report Unit	Total	Anemia		*p*-Value
		Yes	No	
Stunting *n* (%)				
Yes	242 (56.0)	167 (56.8)	75 (54.4)	0.632
No	190 (44.0)	127 (43.2)	63 (45.6)	
Wasting *n* (%)				
Yes	48 (11.1)	33 (11.2)	15(10.9)	0.913
No	384 (88.9)	261 (88.8)	123 (89.1)	
Underweight *n* (%)				
Yes	152 (35.2)	111 (37.8)	41 (29.7)	0.103
No	280 (64.8)	183 (62.2)	97 (70.3)	
Diversified diet in last 24 h *n* (%)				
Yes	133 (39.8)	87 (29.6)	46 (33.3)	0.432
No	299 (69.2)	207 (70.4)	92 (66.7)	

Table A2. Clinical characteristics of children aged 1–5 years in Popokabaka.

	Total	Anemia		*p*-Value
		Yes	No	
Diarrhea in last 2 weeks *n* (%)				
Yes	78 (18.1)	58 (19.7)	20 (14.5)	0.187
No	354 (81.9)	236 (80.3)	118 (85.5)	
Bloody stools in last 2 weeks				
Yes	18 (4.17)	13 (4.4)	5 (3.6)	0.801
No	414 (95.8)	281 (95.6)	133 (96.4)	
Fever in last 2 weeks				
Yes	249 (57.6)	186 (63.3)	63 (45.7)	0.001
No	183 (42.4)	108 (36.7)	75 (54.4)	
Cough in last 2 weeks				
Yes	141 (32.64)	97 (33.0)	44 (31.9)	0.819
No	291 (67.4)	197 (67.0)	94 (68.1)	
Zinc tablets in last 2 weeks				0.649
Yes	23 (5.3)	17 (5.8)	6 (4.4)	
No	409 (94.7)	277 (94.2)	132 (95.6)	
Vitamin A supplements in last 6 months				0.520
Yes	232 (53.7)	161 (54.7)	71 (51.5)	
No	200 (46.3)	133 (45.2)	67 (48.5)	
Deworming in last 6 months				
Yes	306 (70.8)	209 (71.1)	97 (70.3)	0.865
No	126 (29.2)	85 (28.9)	41 (29.7)	

Table A2. *Cont.*

	Total	Anemia		*p*-Value
		Yes	No	
Iron supplements in last 3 months				0.077
Yes	154 (35.6)	113 (38.4)	41 (29.7)	
No	278 (64.4)	181 (61.6)	97 (70.3)	
Sleeping under mosquito nets				0.406
Yes	260 (60.2)	173 (58.8)	87 (63.0)	
No	172 (39.8)	121 (41.2)	51 (37.0)	

Table A3. Prevalence and distribution of anemia, iron deficiency, and malaria.

	n	%	(95% CI)
Anemia			
Anemia, all types (Hgb < 11.0 g/dL)	294/432	68.1	(64.0–72.0)
Severe anemia (Hgb < 7.0 g/dL)	14/432	3.3	(1.9–5.4)
Moderate anemia (Hgb 7.0–9.9 g/dL)	153/432	35.4	(31.0–40.1)
Mild anemia (Hgb 10.0–10.9 g/dL)	127/432	29.4	(25.3–33.9)
Iron Deficiency			
ID as unadjusted ferritin < 12 µg/L when CRP < 5	1/193	0.5	(0.5–1.5)
ID as CRP-adjusted ferritin < 12 µg/L	7/400	1.8	(0.5–3.0)
ID as TIBC > 66 umol/L	33/419	7.9	(5.2–10.5)
ID as TSAT < 20%	55/412	12.9	(9.6–16.1)
Malaria and Inflammation			
Malaria (positive rapid *P. falciparum* test)	375/432	86.8	(83.6–90.0)
CRP < 5 mg/L	207/419	49.4	(44.6–54.0)

Table A4. Spearman correlation of hemoglobin with iron biomarkers, r coefficient (*p*-value).

	Hemoglobin	Ferritin	CRP	TSAT	Serum Iron	TIBC
Hemoglobin	1.000					
Ferritin	−0.17 (0.002)	1.000				
CRP	−0.19 (0.000)	0.35 (0.000)	1.000			
TSAT	−0.02 (0.765)	0.31 (0.000)	−0.08 (0.161)	1.000		
Serum Iron	0.07 (0.170)	0.07 (0.170)	−0.18 (0.000)	0.83 (0.000)	1.000	
TIBC	0.14 (0.011)	−0.51 (0.000)	−0.19 (0.000)	−0.46 (0.000)	0.04 (0.502)	1.000

Table A5. Factors associated with anemia, crude and adjusted odds ratios (ORs) reported.

	Bivariate Estimates			Multivariate Logistic Regression Analysis		
	OR $_{Crude}$	**CI**	***p* Value**	**OR $_{Adjusted}$**	**CI**	***p* Value**
Age	0.99	0.97–1.01	0.505	-	-	-
Household size	1.05	0.96–1.13	0.277	-	-	-
Sex						
Girl	1.01	0.68–1.52	0.927	-	-	-
Boy	1					
Diarrhea in last 2 weeks						
Yes	1.45	0.83–2.52	0.187	1.26	0.63–2.52	0.519
No	1					
Bloody stools in last 2 weeks						
Yes	1.23	0.43–3.52	0.699	-	-	-
No	1					
Fever in last 2 weeks						
Yes	2.05	1.36–3.09	0.001	1.71	1.08–2.70	0.020
No	1					
Deworming in last 6 months						
Yes	1.03	0.66–1.61	0.865	-	-	-
No	1					
Iron supplementation last 3 months						
Yes	1.48	0.96–2.28	0.077	1.49	0.92–2.40	0.102
No	1					
Multiple micronutrients in last 3 months						
Yes	0.86	0.51–1.44	0.576	-	-	-
No	1					
Sleeping under mosquito nets						
Yes	0.83	0.55–1.27	0.406	-	-	-
No	1					
Stunting						
Yes	1.10	0.74–1.65	0.632	-	-	-
No	1					
Wasting						
Yes	1.04	0.54–1.97	0.913	-	-	-
No	1					
Underweight						
Yes	1.43	0.93–2.21	0.103	1.37	0.73–2.54	0.321
No	1					
Dietary diversity						
Yes	0.84	0.54–1.30	0.432	-	-	-
No	1					
CRP						
>5 mg/L	1.68	1.10–2.54	0.015	1.65	1.05–2.59	0.029
≤5 mg/L	1					
ID by TSAT						
Yes	0.62	0.34–1.12	0.116	0.50	0.27–0.97	0.038
No	1					
Malaria						
Yes	5.02	2.78–9.05	<0.001	4.08	2.18–9.68	<0.001
No	1					

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Paper II

nutrients

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Article

Distribution and Determinants of Serum Zinc, Copper, and Selenium Levels among Children under Five Years from Popokabaka, Democratic Republic of Congo: A Cross-Sectional Study

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Abstract: Information about essential trace elements among children in many African countries, including the Democratic Republic of Congo (DRC), is limited. We aimed to measure the distribution and determinants of serum zinc (Zn), copper (Cu), and selenium (Se) concentrations in a representative sample of children under five years old. We conducted a community-based cross-sectional study in Popokabaka, DRC. Blood samples were drawn from 412 children. The serum concentrations of minerals were measured using inductively coupled plasma–mass spectrometry. The median concentrations (P25–P75) of Zn, Cu, and Se were 61.9 µg/dL (52.8–70.2), 145.5 (120.0–167.0) µg/dL and 5.3 (4.3–6.3) µg/dL. The CRP-adjusted prevalence of serum Se deficiency was 84.1% (95% confidence interval [CI] 81.4–87.0) and of Zn deficiency was 64.6% (95% CI 59.8–69.1%). Only a few children were Cu deficient [1.5% (0.6–3.2)]. Evidence of inflammation (C-reactive protein, >5 mg/L) was associated with a lower Se concentration and higher Cu concentration. Furthermore, serum Se concentration was positively associated with linear growth. The average Cu/Zn molar ratio (2:1) was twice that recommended. Children in western Popokabaka had higher Zn and Se levels than their eastern neighbors. Zinc and selenium deficiencies are common among children in Popokabaka and require attention and prioritization.

Keywords: zinc; copper; selenium; children; Popokabaka; deficiency

Citation: Mbunga, B.K.; Engebretsen, I.M.S.; Strand, T.A.; Gjengdal, E.L.F.; Akilimali, P.Z.; Langfjord, M.M.; Tugirimana, P.L.; Mapatano, M.A. Distribution and Determinants of Serum Zinc, Copper, and Selenium Levels among Children under Five Years from Popokabaka, Democratic Republic of Congo: A Cross-Sectional Study. *Nutrients* **2022**, *14*, 683. https://doi.org/10.3390/nu14030683

Academic Editor: Anna Kipp

Received: 2 January 2022
Accepted: 3 February 2022
Published: 6 February 2022

1. Introduction

Zinc (Zn), copper (Cu), and selenium (Se) are essential minerals [1] because they generally serve as modulators in various chemical, biological, catalytic, and metabolic processes [2], and play a crucial role in optimal growth and cognitive development during childhood [3]. Furthermore, they are all critical co-factors for the immune system and function in antioxidant-stress modulation. In addition, Zn specifically stabilizes the DNA structure and mediates its replication [4]. Selenium plays a specific role in thyroid function, and its deficiency is associated with thyroid diseases [5]. Copper is involved in erythrocyte differentiation, and its deficiency may cause hypochromic microcytic anemia [6].

As nutrients, they are obtained from food, water, and the environment [7] and are usually needed in tiny quantities (traces) in the body [8,9]. Their toxicities are rare at the dietary intake level, as they are highly regulated by homeostatic mechanisms to avoid excessive

levels and cannot be stored in the body [1]. Deficiencies result from inadequate dietary intake, malabsorption, or other conditions causing intestinal loss [10]. According to the World Health Organization (WHO), deficiencies of these minerals are widespread and contribute to childhood morbidity and mortality, mainly through impaired immunity [11,12].

Although regional projections [13–15] predict the severity of deficiencies of the essential trace elements (TEs) in the sub-Saharan regions, documented information on serum Zn, Cu, and Se concentrations in children from this region is limited. Moreover, the national-level data are limited, probably due to their perceived high cost and logistical challenges [15]. In addition, the recent recognition of inflammation's role in influencing mineral balance [16–19] limits their accurate interpretation, particularly in populations with a high burden of infections. Thus, nutritional assessment of essential minerals should consider exploring inflammatory health conditions affecting the concentrations [20].

In the Democratic Republic of Congo (DRC), reliable information on the burden of Zn, Cu, and Se deficiencies in the normal population is limited. Harvey-Leeson et al. reported a high prevalence of Zn deficiency among children of the Kivu and Kongo Central provinces [21]. Musimwa et al. [22], in the Lubumbashi region, compared the TE levels of severely malnourished children and well-nourished ones, and found that the former had lower TE levels. Bumoko et al. [23], in the rural area of Kahemba, found much lower serum Se, Zn, and Cu levels in Konzo children, and that Se status was positively associated with neurodevelopment.

Considering the unchanged stunting trend and the high infant morbidity and mortality rates in the Democratic Republic of Congo, factors that can impair health and growth, such as Zn, Cu, and Se status in a representative sample of children, should be identified. Therefore, this study aimed to describe the serum Zn, Cu, and Se concentrations and identify their determinants among the children in Popokabaka, DRC.

2. Materials and Methods

2.1. Design and Study Location

The present analysis is part of a population-based cross-sectional study conducted between May and June 2019 in the Popokabaka health zone, Kwango Province (formerly Bandundu Province), DRC. This region (5°22′49.26″ S–5°25′48″ S, 16°20′26.16″ E–16°22′14.88″ E) is known for the poor soil and food insecurity. It is an entirely rural region without a known mining history, but is close to the Kahemba region and Angola border, known for their notorious diamond mining exploitation. The Kwango River, which takes its source in Angola and crosses the mining region, divides the Popokabaka region into two. Agriculture, based on monoculture, remains the primary source of income for the community. People prefer to grow cassava as their staple food because of its resistance to soil drought, marketable roots, and culturally accepted leaves. Konzo, a neurotoxic motor disease, is prevalent in the region, mainly affecting children and women [15–18]; it is a disease explained by incomplete preparation of bitter cassava. The people in Popokabaka have limited access to agricultural fertilizers. Livestock and poultry are less practiced due to the lack of drugs to fight epidemics in animals. People avoid fishing due to their fear of crocodiles in the Kwango River. Animal-sourced foods are, thus, rarely consumed, and accessibility for imported foods and goods from other towns is also limited because of poor roads. People in Popokabaka drink untreated water from groundwater sources. Chronic malnutrition is common because half of all children are stunted [24,25]. Malaria and anemia are also highly prevalent [26].

2.2. Participants and Sampling

A total of 432 children aged 12–59 months were included in this study. As the present research is part of a multiple-outcomes biomarker survey, the calculation was based on a proportional sample size calculation for anemia prevalence of0.59, a precision of 0.075, and a design effect of two. Children were selected using a three-stage cluster sampling technique. More details on sampling are described in our former article [26].

The flow chart in Figure 1 shows the recruitment and sample exclusion conducted in the present study. Children belonged to five health areas: Kabangu, Ingasi, Cite Popo, Secteur Popo, and Tzunza. Four of them are on the eastern side, and one is on the western side of the Kwango River.

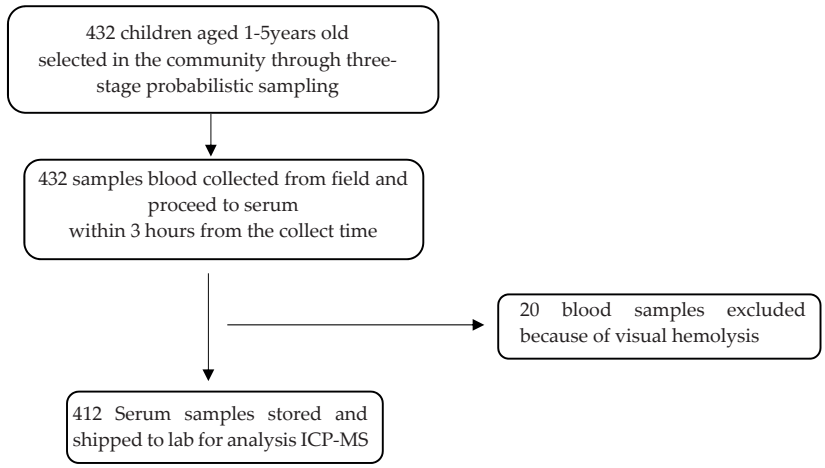

Figure 1. Flowchart of participants recruitment.

2.3. Data Collection Technique

Data collection was organized in 2 consecutive days in each cluster: a household survey with anthropometry on the first day and blood collection on the following day. More details can be found in our previous paper [26]. Anthropometric indices including weight-for-height, height-for-age, weight-for-age, and mid-upper-arm-circumference-for-age, and their Z Scores, were calculated using WHO Anthro software.

2.4. Blood Processing and Management

On the second day, phlebotomists first performed a capillary finger-prick test for hemoglobin (Hgb) assessment (Hemocue 301) and a rapid test for malaria in the field. Then, they collected up to 6 mL of venous blood from 432 children aged 1–5 years. The serum was separated from the blood cells within three hours. We used trace-element-free equipment and techniques previously described to ensure that samples were not contaminated [26]. We excluded blood samples ($n = 20$) with visual hemolysis or insufficient quantity. The separation was performed at 2300 rpm for 10 min (RCF 1532 g) using a Hettich centrifuge (Tuttlingen, Germany). The serum was aliquoted into two polypropylene vials: 0.5 mL tricoded FluidX vials (Brooks Life Science) and 2 mL Sarstedt vials (Sarstedt, Nümbrecht, Germany). We stored the vials, frozen at −40 °C, until the survey ended. Then, we transported all samples in liquid nitrogen from Popokabaka to Kinshasa (a 12 h vehicle trip). The vials were transferred to −80 °C ultra-low freezers at Kinshasa School of Public Health for a week. Then, vials were shipped on dry ice and under continuous temperature monitoring to two labs in Norway. All of the 0.5 mL vials were sent to Haukeland University Hospital (Bergen, Norway) for the analysis of serum ferritin (using an electrochemiluminescence immunoassay (ECLIA)), C-reactive protein (S-CRP) (using the immunoturbimetry method), and total iron-binding capacity (S-TIBC, using the Berekna equation calculation: s-TIBC = s-Transferrin * 25.1). Two-milliliter vials were sent to the Norwegian University of Life Sciences (Ås, Norway) for the analysis of Zn, Cu and Se (using the Agilent 8900 Triple Quadrupole inductively coupled plasma–mass spectrometer (ICP-MS)). Transferrin saturation (TSAT), expressed as a percentage, was then calculated as the value of serum iron divided by the TIBC.

2.5. Sample Preparation for Mineral Analysis

Using a 100–1000 µL pipette (Sartorius, Göttingen, Germany) and Thermo Scientific ART Barrier pipet tips (Waltham, MA, USA), 250-µL aliquots of thawed, tempered, and homogenized serum was transferred into 5 mL polypropylene tubes (Sarstedt, Nümbrecht, Germany) and accurately weighed (Sartorius MC 210P). Subsequently, using a 10–300 µL electronic pipette (Biohit, Helsinki, Finland), 100 µL of internal standard (rhodium [Rh] and selenium [74Se]) and 500 µL of nitric acid (HNO3, 69% weight [w]/w, sub-boiled ultra-pure) were added to each sample before digesting for 3 h at 90 °C in a heating cabinet (Termaks, Bergen, Norway). Finally, the samples were diluted to 5.00 mL with deionized water (>18 MΩ). To stabilize mercury in the solution, 100 µL hydrochloric acid (HCl, 37% *w*/*w*, sub-boiled ultra-pure) was added to each sample.

2.6. Sample Analysis

The total element concentrations in the serum were quantified by inductively coupled plasma–mass spectrometry using the Agilent 8900 Triple Quadrupole (QQQ) ICP-MS. The masses were (Q1/Q2): Cu (63/63) and Zn (66/66) with gas-mode ammonia (NH3) and Se (78/94), using gas-mode oxygen (O2). Detection and quantification limits were calculated by multiplying the standard deviations of the blank samples (n = 10) by three and ten, respectively. The ten blank samples were taken through the whole measurement procedure, including the sample preparation steps. The limit of detection (LOD) and limit of quantification (LOQ) were determined in µg/dL as Cu (0.1/0.49), Zn (6/20), and Se (0.08/0.27), respectively.

2.7. Quality Control

We assessed the blank samples for contamination of reagents and the equipment used. The accuracy was evaluated by concurrent analysis of SeronormTM Trace Elements, Serum L1 and L2 (Billingstad, Norway). The data obtained were within a 95% confidence level of the certified values issued. The method's "within-laboratory reproducibility" (RSD) was <1.4% for Zn, Cu, and Se; the results were obtained by measuring 12 replicate samples of the serum on three different days.

2.8. TE Threshold Definitions

We defined deficiency at a level of Cu < 80 µg/dL, Zn < 65 µg/dL [27], and Se < 7.0 µg/dL [10]. Anemia was defined as Hgb levels <11 g/dL, and Iron Deficiency was set for transferrin saturation <20%. To account for inflammation [28], the regression-correction approach developed by BRINDA was used for minerals that correlated with CRP using the following equation: Adjusted mineral = unadjusted mineral—β (CRPobs–CRPref). First, we defined internal reference values for inflammatory markers (CRPref) as the tenth percentile [29]. Then, the regression coefficient (β) for the association was estimated between CRP and each mineral value using univariable linear regression models, with the minerals as dependent variables.

2.9. Statistical Management

Data were analyzed using Stata 16.1 (StataCorp LLC, College Station, TX, USA). First, we investigated the normal distribution of TE concentrations using the Kolgomorov–Smirnov test. None of TEs were normally distributed. Statistics were then summarized as medians with 25th and 75th percentiles (P25–P75). The prevalence was reported as proportions with their 95% confidence intervals (95 CI). We also calculated the Cu/Zn ratio. Then, the Mann–Whitney and Kruskal–Wallis tests were performed to compare mineral concentrations across categorical variables, whereas Spearman's rank–order test coefficient was used to assess any correlation among continuous variables. A simple linear regression analysis was used to adjust mineral concentrations for inflammation (CRP). Bivariate and multiple linear regression analyses were performed to identify significant determinants for every mineral. Then, we reported the unadjusted and adjusted regression coefficients,

95% CI, and corresponding *p*-values. The final regression models were constructed following a forward stepwise selection approach with covariate inclusion probability (*p*-value of crude odds ratio) of <0.20. Multicollinearity, moderation and mediation effects were checked for all of the regression models.

3. Results

3.1. Characteristics of the Study Population

The serum was collected from 412 children aged 1–5 years. The boy/girl ratio was 1:1, and their median (P25–P75) age was 32 [22–43] months. Table 1 shows the general characteristics of the children included in this study. Stunting was the most common nutritional problem observed in 55.3% of the children, whereas one in ten children (10.7%) was wasted. From the two preceding weeks, half of the children (58.0%) had experienced symptoms such as diarrhea (17.2%) and cough (32.3%). This inflammatory status was also confirmed in 49% of the children who had an elevated CRP level. Anemia was prevalent in 68% of the children, and iron deficiency was not equally common (7.5%). Under this tropical location, malaria was the leading health problem, and was found in nine in ten children (86.9%). Many of the children had received nutritional supplements, such as Zn (5.1%) and iron (35.7%), two weeks before the study initiation.

Table 1. General characteristics of the population in a cross-sectional study on the micronutrient status in children from Popokabaka.

Characteristics	N (412)	%
Age [median (P25–P75)]	32 (22–43)	
Sex		
Boy	212	51.5
Girl	200	48.5
Stunting	228	55.3
Wasting	44	10.7
Underweight	140	34.0
Fever in the two preceding weeks	239	58.0
Diarrhea in the two preceding weeks	71	17.2
Cough in the two preceding weeks	133	32.3
Anemia	280	68.0
Iron deficiency	53	12.9
Iron-deficiency anemia	31	7.5
Inflammation state (elevated CRP)	202	49.0
Malaria by rapid test	358	86.9
Zinc supplementation in the two preceding weeks	21	5.1
Iron supplementation in the two preceding weeks	147	35.7
Micronutrient powders in the two preceding weeks	77	18.7

3.2. Distribution of Serum Zn, Cu, and Se

The serum Zn, Cu, and Se concentrations are shown in Table 2 as medians (P25–P75). The concentrations of these nutrients were not associated with age or sex. Acute inflammation status was negatively associated with Zn and Se levels, and positively associated with Cu levels. Children with elevated CRP levels (>5) had lower Zn and Se levels than those with normal CRP levels (p-value < 0.001). In contrast, children with elevated CRP levels had higher Cu levels than those with normal Cu levels (p-value < 0.001). Among the three minerals, only Se was associated with stunting and anemia. Children with stunting (HAZ Z Score < –2) or anemia (Hgb < 11 g/dL) were more likely to have a more profound Se deficiency. Having malaria was not associated with any of the micronutrient concentrations.

Table 2. Distribution of serum Zn, Cu, and Se as median (P25–P75) over different groups of children.

	n	Zn µg/dL	Cu µg/dL	Se µg/dL
Total	412	61.9(52.8–70.2)	145.5 (120.0–167.0)	5.3 (4.3–6.3)
Child sex				
Male	212	61.8 (52.3–70.5)	150.0 (125.0–173.0)	5.2(4.1–6.3)
Female	200	62.4 (52.9–70.1)	142.0 (116.0–162.5)	5.4 (4.4–6.3)
p-value		0.816	0.093	0.403
Age group				
12–23	117	61.7 (53.3–71.6)	146.0 (124.0–170.0)	5.4 (4.5–6.3)
24–35	115	61.8 (52.8–69.0)	143.0 (113.0–166.0)	5.2 (4.1–5.9)
36–47	110	63.3 (53.2–70.8)	151.5 (131.0–169.0)	5.3 (4.8–6.4)
48–59	70	60.6 (52.0–71.0)	139.0 (110.0–163.0)	5.3 (3.7–6.2)
p-value		0.826	0.099	0.442
Anemia				
No	132	63.4 (54.2–73.0)	147.0 (118.5–164.5)	5.5 (4.7–6.4)
Yes	280	60.9 (52.1–68.8)	145.0 (120.5–170.5)	5.2 (4.1–6.1)
p-value		0.220	0.240	0.033
Iron deficiency				
No	359	62.0 (53.7–70.7)	144.0 (117.0–166.0)	5.3 (4.3–6.3)
Yes	53	60.1 (49.5–69.8)	150.0 (131.0–173.0)	5.5 (4.1–6.4)
p-value		0.043	0.019	0.918
Stunting				
No	184	62.5 (53.0–72.9)	146.0 (120.0–173.0)	5.4 (4.5–6.6)
Yes	228	61.5 (52.7–68.4)	144.0 (119.0–164.0)	5.2 (3.9–6.0)
p-value		0.293	0.456	0.004
Inflammation state				
No	210	63.3 (56.7–71.9)	135.0 (111.0–155.0)	5.6 (4.8–6.7)
Yes	202	59.5 (50.2–67.7)	155.5 (136.0 – 181.0)	5.0 (3.8–5.8)
p-value		<0.001	<0.001	<0.001
Malaria				
No	54	64.0 (55.1–75.5)	152.0 (133.0–173.0)	5.4 (4.6–5.9)
Yes	358	61.4 (52.6–68.9)	145.0 (119.0–166.0)	5.3 (4.3–6.4)
p-value		0.140	0.533	0.415
Kwango river side				
East	343	60.2 (52.2–68.2)	146.0 (120.0–168.0)	5.2 (4.1–5.0)
West	69	67.3 (61.9–74.8)	145.0 (119.0–163.0)	6.2 (5.2–7.0)
p-value		<0.001	0.976	<0.001

Considering that the Kwango River completely halves the Popokabaka health zone, children from villages on the eastern side of the river were found more deficient in Se and Zn than those from villages on the western side.

3.3. Prevalence of Deficiencies of Zn, Cu, and Se

Zn and Se deficiencies were widespread among children in Popokabaka (see Table 3), whereas Cu deficiency was found only in 6 of the 412 (1.5%) children. Se deficiency prevalence was severe in 86.9% (95 CI 83.3–89.8) of the children, without considering inflammation. Inflammation adjustment lowered the Se deficiency prevalence to 84.1 % (81.4–87). Zn deficiency was observed in 64.6% (95 CI 59.8–69.0) of the children.

Table 3. Prevalence of Zn, Cu, and Se deficiencies among 1–5-year-olds in Popokabaka.

	Unadjusted Prevalence % (95 CI)	CRP-Adjusted Prevalence % (95 CI)
Zn deficiency	64.6 (59.8–69.0)	64.6 (59.8–69.0)
Cu deficiency	1.5 (0.6–3.2)	1.5 (0.6–3.2)
Se deficiency	86.9 (83.3–89.8)	84.1 (81.4–87.0)

3.4. Correlations between the Biomarkers

Bivariate correlations between the different biomarkers are shown in Table 4; this table reveals that the serum Zn levels were positively correlated with serum Cu and Se levels. Simultaneously, no relationship was observed between the serum Cu and Se levels. The Cu/Zn ratio, known for its association with cognitive functioning in early life, scored 2:1, twice more the standard accepted. Second, the serum Zn and Se levels were positively correlated with hemoglobin levels, whereas Cu was negatively correlated with transferrin saturation (TSAT). In other words, the lower the Zn or Se level in children, the lower the hemoglobin level. Moreover, the higher the Cu level, the higher the occurrence of iron deficiency. Finally, Zn and Se concentrations were negatively correlated with CRP level, whereas Cu was positively correlated with CRP.

Table 4. Spearman correlation coefficient between continuous variables.

	Zinc	Copper	Selenium
Copper	0.23 ***	1.000	0.35 ***
Selenium	0.35 ***	0.07	1.000
Height-for-Age Z Score	0.07	0.05	0.15 **
Weight-for-Age Z Score	−0.05	−0.03	0.00
Household food-insecurity access score	−0.14 **	−0.09	0.05
Hemoglobin	0.14 **	−0.06	0.17 **
Transferrin saturation	−0.00	−0.12 **	−0.06
C-reactive Protein	−0.24 ***	0.39 ***	−0.33 ***

** p-value < 0.01; *** p-value < 0.001.

3.5. Determinants of Serum Zn, Cu, and Se

The Table 5 is showing determinants of serum Zn, Cu, and Se concentrations. Regarding Zn, only the side of Kwango River on which children were located determined the serum Zn levels. Children living in villages on the western side had higher serum Zn than those from the eastern side (increased by 5.95). Regarding Cu, the inflammatory state was a powerful determinant that positively determined Cu levels in the children of Popokabaka. With inflammation (CRP $\geq$ 5 mg/dL), the serum Cu level increased by 23 µg/dL. Transferrin saturation negatively determined the Cu level, indicating that the more iron deficient (TSAT decreasing) the child was, the higher his/her Cu level was. Conversely, children with higher Cu levels had been administered iron tablets in the two previous weeks. Girls had less Cu than boys. Selenium was positively related to the height-for-age Z Score, indicating that the more stunted the child was, the more Se-deficient he or she was. Consistent with Zn, serum Se was more concentrated in children on the left side of Popokabaka. Inflammation negatively determined the Se level, even after adjustments.

Table 5. Multiple linear regression analysis and β coefficients of determinants of serum zinc, copper, and selenium levels.

	Zn-Model				Cu-Model				Se-Model			
	Crude Estimates		Adjusted Estimates		Crude Estimates		Adjusted Estimates		Crude Estimates		Adjusted Estimates	
	β	95% CI	β	95% CI	B	95% CI	β	95% CI	B	95%CI	β	95% CI
Sex girl	1.55	[−2.07; 5.17]	-	-	−10.02 [a]	[−17.47; −2.57]	−8.32 [b]	[−15.37; −1.26]	0.08	[−0.22; 0.37]	-	-
Age	0.06	[−0.08; 0.21]	-	-	−0.32 [a]	[−0.62; −0.03]	−0.26	[−0.54; 0.02]	0.00	[−0.01; 0.01]	-	-
Diarrhea	−4.50 [a]	[−9.27; 0.28]	−4.36	[−9.11;0.40]	−4.57	[−14.50; 5.36]	-	-	−0.19	[−0.58; 0.20]	-	-
Zinc supplementation	2.56	[−5.67; 10.78]	-	-	8.62	[−8.42; 25.68]	-	-	0.38	[−0.30; 1.04]	-	-
Fever	0.52	[−3.14; 4.19]	-	-	5.99 [a]	[−1.60; 13.57]	1.93	[−5.32; 9.18]	−0.14	[−0.44; 0.16]	-	-
Cough	0.06	[−3.81; 3.93]	-	-	3.38	[−4.64; 11.40]	-	-	−0.26 [a]	[−0.57; 0.06]	−0.21	[−0.50; 0.09]
Iron supplementation	−0.68	[−4.45; 3.10]	-	-	9.89 [a]	[2.12; 17.67]	8.25 [b]	[0.86; 15.63]	−0.06	[−0.37; 0.25]	-	-
Micronutrient powder	−1.45	[−6.09; 3.19]	-	-	−0.80	[−10.43; 8.82]	-	-	−0.08	[−0.46; 0.30]	-	-
Elevated CRP	−2.29	[−5.90; 1.32]	-	-	24.5 [a]	[17.4; 31.64]	23.4 [b]	[16.30; 30.45]	−0.76 [a]	[−1.05; −0.47]	−0.68 [b]	[−0.95; −0.40]
Height-for-Age Z Score	−0.27	[−0.82; 1.35]	-	-	0.37	[−1.87; 2.62]	-	-	0.13 [a]	[0.04; 0.22]	0.12 [b]	[0.04; 0.20]
Weight-for-Age Z Score	−1.02 [a]	[−2.45; 0.42]	−0.94	[−2.37; 0.48]	−0.56	[−3.54; 2.42]	-	-	−0.04	[−0.15; 0.08]	-	-
HFIAS	−0.27 [a]	[−0.55; 0.02]	−0.25	[−0.53; 0.04]	−0.38	[−0.97; 0.22]	-	-	0.00	[−0.02; 0.03]	-	-
Malaria	−1.89	[−7.25; 3.47]	-	-	−5.57	[−16.68; 5.54]	-	-	0.15	[−0.29; 0.59]	-	-
Hgb	−0.04	[−0.46; 0.38]	-	-	−0.34	[−1.21; 0.54]	-	-	0.00	[−0.03; 0.04]	-	-
Transferrin saturation	0.02	[−0.02; 0.05]	-	-	−0.08 [a]	[−0.15; −0.02]	−0.08 [b]	[−0.14; −0.02]	0.00	[−0.00; 0.00]	-	-
Western side river	6.20 [a]	[1.39; 11.0]	5.93 [b]	[1.13; 10.74]	−2.95	[−13.00; 7.10]	-	-	1.13 [a]	[0.76; 1.52]	1.04 [b]	[0.67; 1.41]

[a] p-value < 0.20 for inclusion in the model; [b] p-value < 0.05 for determinant significance.

4. Discussion

The present study was conducted in the context that there is a lack of community-based data on serum levels of essential TEs. In a representative sample of children aged <5 years from the Popokabaka region, we assessed serum Zn, Cu, and Se levels and identified determinants of these minerals. In short, Zn and Se deficiencies were severely prevalent, and normal Cu levels were observed, implying a mineral imbalance (Cu/Zn ratio as 2:1) potentially contributing to cognitive development and growth impairments. The study also determined some factors that possibly explain the variation of serum Zn, Cu, and Se levels, and that could guide decisions and policy making.

4.1. Zn Deficiency

Zn is necessary for adequate growth, immunocompetence, and cognitive development [30,31]. Using Zn availability indicators in the food supply database and a stunting indicator, Wessells et al. [15] predicted a severity (>40%) of Zn deficiency risk for the DRC region. Accordingly, 64% of children were Zn deficient in our study. Children of Popokabaka lack Zn, like children in other sub-Saharan countries [14]. However, our observations in Popokabaka (Kwango Province) seem to be more severe than the ones from the Kongo Central and south Kivu provinces in DRC (25–29%), as reported by Harvey-Leeson in 2016 [21]. This remarkable difference could be explained by the difference in dietary habits and the better food availability and diversity observed in the Kongo Central and south Kivu regions than in Popokabaka [32]. For instance, communities in the Kivu province are pastoral, commonly practice livestock, and have great accessibility to meat, beans, and potatoes, which are culturally accepted as staple foods. The Kongo Central province also has varied food availability because of its geopolitical situation as the entrance for food importation from the Atlantic Sea. Thus, dietary customs are diversified with possible high Zn intake in these communities. By contrast, Popokabaka is enclaved, entirely rural, hard to reach, and almost inaccessible for food importation from big towns in DRC. Crop variety and animal-sourced foods are limited, and poverty hurts the population, as evidenced by the high level of Se and Zn deficiency. As is already known, food accessibility contributes to increasing dietary diversity and nutritional intake in a community [33]. These studies imply high variation and disparities in serum Zn deficiency across DRC provinces, and further national-scale surveys should be considered in DRC. Moreover, location remained the only stable determinant in our Zn model. Those living on the western side of the Kwango River had higher serum Zn levels. This reinforces the hypothesis of geographical disparities of Zn across regions, although the spatial analysis was not performed to confirm the spatial dependency of Zn deficiency, as established in the literature [34,35].

The negative effect of inflammation status on serum Zn levels observed in bivariate analysis comes to null after adjusting other covariates in the multivariate regression model. This observation is in discordance with the literature that supports a stable negative determination of inflammation [16,21,36–38]. We know that in inflammatory conditions, Zn moves from plasma to the liver in the production of pro-inflammatory molecules, lowering its plasma or serum level. This effect was suppressed with our data, probably because only the acute phase was considered using the CRP as a biomarker. We did not measure the Alpha-globulin glycoprotein, which better reflects chronic inflammation, as there would be a classification bias that distorted the true expected effect. Again, Zn was not determined by inflammatory-related diseases (diarrhea or cough in the last two weeks) in our data, although other studies indicate a reduction effect of zinc on morbidity and mortality for childhood diarrhea and pneumonia [39–42] and adult COVID-19 [43]. We hypothesize that other Zn influencing factors (Zn dietary intake, Zn absorption, and the ratio of phytate/Zn) should be considered in a further Zn model. In addition, unpublished documentation from Popokabaka health management reports high diarrhea incidence, justifying the systematic use of Zn supplements as an adjunct therapy for diarrhea in Popokabaka health facilities.

As previously observed, stunting prevalence, an indirect indicator of Zn deficiency [10], is also severe in Popokabaka. The coexistence of severe stunting, Zn deficiency, and Se defi-

ciency in Popokabaka suggests stable, permanent, and long-term essential TE deficiencies that require specific food-based strategies [44].

Zn is known to contribute to growth. However, we found no association with the height-for-age Z Score. This observation is also in line with several observational studies [45–50]. The serum Zn level is contextual, changing, and highly influenced by the moment and dietary intake, and may not be explained by stature retardation, which has occurred in the early stages of life. Moreover, the cross-sectional design does not allow any prospective assessment of Zn and growth.

4.2. Cu Deficiency, Cu-Deficiency Anemia, and Cu/Zn Ratio

The present study reported a normal serum Cu concentration among children of Popokabaka, with 1.5% Cu deficiency prevalence, without being related to anemia [39], but a negative correlation with transferrin saturation. This imbalance may result from increased circulating ceruloplasmin as a stress response, resulting from inadequate hemoglobin synthesis or inflammation. Ceruloplasmin, also known as ferroxidase, contributes to the homeostatic regulation of both minerals in the serum. It binds to approximately 95% of serum Cu and oxidizes ferrous iron [3].

Cu, a bivalent mineral, is also an antagonist Zn. A Zn shift from the plasma into the liver during the inflammation response tends to increase Cu as plasma Zn decreases. Other studies [51] also reported such a mineral imbalance and normal Cu distribution with high Zn deficiency. In addition, the Cu/Zn ratio is a valuable parameter for a range of detrimental health conditions: increased Cu/Zn ratio may indicate growth impairment, cognitive abnormalities, risks of bowel diseases, and increased oxidative stress and cardiovascular disease [18,52]. We reported a Cu/Zn ratio of 2:1, which was twice the recommended standard (1:1), implying a need for an urgent Zn strategy (Zn fortification or supplementation) to increase Zn, thereby correcting the Cu/Zn ratio among children of Popokabaka.

4.3. Se Deficiency

Considering a lesser-known nutrient, Se plays a role in DNA reproduction, metabolism, synthesis, and protection from oxidative damage caused by free radicals [3]. For the first time in the DRC, the present study reported the prevalence of serum Se deficiency from a representative population of children. Eight in ten children in Popokabaka (exactly 84.1%) had Se deficiency. This severity is consistent with the prediction of Se risk deficiency (76–90%, for DRC) based on indirect indicators (Se supplies in food systems) for the sub-Saharan regions, reported by Joy et al. in 2014 [53]. Humans take Se from the soil and water through plants and seafood. Living in an area with low Se content in the soil exposes us to a high risk of Se-deficient blood.

Similarly, Ligowe et al. in 2019 established a geospatial linkage between the risk of Se deficiency and sub-Saharan food systems, mainly through limited access to animal sources. They reported that the Se level in individuals is typically dependent upon the region one lives in and the foods they consume. Dietary customs or habits may also influence Se intake. The population with limited accessibility to the best sources of Se (meat, fish, and eggs) may be at higher risk of Se deficiency [54].

Diets in Popokabaka are mainly based on cassava starch and leaves, which lack Se and selenoproteins (selenomethionine and selenocysteine). The consumption of such a staple food without significant animal food sources may limit the Se intake and its tissue stock. Se deficiency has been associated with some health outcomes. Ngo et al. [55] observed that Se deficiency was associated with iodine deficiency and cretinism in women with endemic myxedematous goiter in DRC (ancient Zaire), including Popokabaka. Although iodine disorder diseases (IDDs) have been almost eliminated through the successful iodine–salt fortification strategy, the presence of a possible iodine deficiency should be considered in our study area. Popokabaka is an ancient endemic goiter area with unknown Se intervention. Despite IDDs, Bumoko et al. [23] found a lower serum Se level in children with Konzo disease and a significant association between Se deficiency and cognitive performance

parameters. Konzo is also prevalent in the Popokabaka region [56–59] and has, like IDDs, been associated with cyanate precursors that remain highly concentrated in inappropriately processed cassava plants. Food-based strategies, which are most commonly recommended in the case of population Se deficiency, should consider the cyanate metabolic patterns that are implicated these health outcomes. Mass Se supplementation, observed as a marginal strategy because of its overdose consequences, should not be recommended in this case.

4.4. Limitations and Strengths

Our cross-sectional study identified some factors as possible determinants of serum Zn, Cu, and Se levels among children in Popokabaka. However, temporal relationships between these factors (exposures) and outcome variables of interest cannot be ensured; thus, causality cannot be confirmed.

In addition to the representativeness of the population of Popokabaka children, our study has the strength of providing community-based information on the serum level of multiple essential TEs from this rural hard-to-reach area for the first time. Our results are generalizable to the larger child population of Popokabaka, and deficiencies we have reported might be expected for similar rural regions in DRC. Furthermore, repeatable handling procedures were tested to manage contamination risk and cold chain challenges in such complex research conditions.

5. Conclusions

Mineral levels in the body depend on the population's characteristics and its diet, geographic area, and soil composition. We report that the serum Se and Zn concentration in children under five years old, residing in Popokabaka, is significantly below standard cut-off values, thus indicating a severe dietary deficiency of these TEs. A cost-effective and sustainable intervention to address the Se and Zn status in Popokabaka now emerges as a research priority.

Author Contributions: Original draft preparation, B.K.M.; conceptualization, B.K.M., M.A.M., T.A.S. and I.M.S.E.; standard operating procedure development, P.L.T., E.L.F.G. and M.M.L.; fieldwork supervision, B.K.M., M.A.M. and I.M.S.E.; data analysis, B.K.M., P.Z.A., and T.A.S.; writing—review and editing, all coauthors; project administration, M.A.M. and I.M.S.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Norwegian Agency for Development (NORAD) and cooperation, the Norwegian Higher Education (NORHED) growing partnership for higher education and research in nutrition (GrowNut Project: QZA-0484, COG-13/0002) in the DRC, and a collaboration between the University of Kinshasa, the University of Kwazulu-Natal, and the University of Bergen.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Norwegian Institutional Review Board '' REK Committee (ref: 2018/1420/REK vest, date: 30 November 2018) and the Kinshasa School of Public Health ethical committee (ref: ESP/CE/2019, date: 28 January 2019), also in Bergen. Other authorizations were requested from both the local administrative and health authorities.

Informed Consent Statement: Written informed consent was obtained from the mothers or caretakers of children in this study.

Data Availability Statement: The dataset of this study can be made available on reasonable request to B.K.M.

Acknowledgments: The authors thank the participants and all of the community members of Popokabaka for collaboration and participation in this study.

Conflicts of Interest: The authors declare no conflict of interest.

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Paper III

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Heavy metals in children's blood from the rural region of Popokabaka, Democratic Republic of Congo: a cross-sectional study and spatial analysis

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Exposure to heavy metals can affect cell differentiation, neurocognitive development, and growth during early life, even in low doses. Little is known about heavy metal exposure and its relationship with nutrition outcomes in non-mining rural environments. We carried out a community-based cross-sectional study to describe the distribution of four heavy metal concentrations [arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg)] in the serum of a representative population of children aged 12 to 59 months old from the rural region of Popokabaka, Democratic Republic of Congo. The four metals were measured in 412 samples using inductively coupled plasma–mass spectrometry (ICP–MS). Limits of detection (LoD) and quantification (LoQ) were set. Percentiles were reported. Statistical and geospatial bivariate analyses were performed to identify relationships with other nutrition outcomes. Arsenic was quantified in 59.7%, while Cd, Hg, and Pb were quantified in less than 10%, all without toxicities. The arsenic level was negatively associated with the zinc level, while the Hg level was positively associated with the selenium level. This common detection of As in children of Popokabaka requires attention, and urgent drinking water exploration and intervention for the profit of the Popokabaka community should be considered.

Regardless of multiple controversial definitions[1], heavy metals (HMs) are commonly characterized as chemical elements with relatively high atomic numbers and densities (more than 20 and more than 5 g/cm^3, respectively) that are naturally found on Earth and that vary geographically from one region to another[2]. In a place with a high concentration of HMs, they easily enter the food systems and contaminate all food products (including water) from the Earth in a cycling chain that could cause harmful effects on consumers[3,4]. These metals are of public health interest because of their direct harmful effects on human health[5,6] and indirect interactions with other essential minerals[7]. They may cause poisoning and serious irreversible health effects even at low doses[8–11]. During pregnancy, exposure to heavy metals can be neurotoxic and may impair child development[12]. During infancy, additional routes of exposure, including breastfeeding and high-risk behaviors, such as hand-to-mouth activities, make children vulnerable to HM poisoning[13,14].

Among the various HMs, arsenic (*As*), cadmium (*Cd*), lead (*Pb*), and mercury (*Hg*) are the most common and are considered harmful[9–11]. Arsenic is widely distributed in natural waters, and groundwater is one of the primary routes of exposure to inorganic *As*[15]. Long-term exposure to inorganic *As* during infancy increases the risk of lower respiratory tract infection, gastrointestinal illnesses, and cancer[15,16]. Lead, which has no physiological role in humans, is frequently found in household dust. More than 95% of the total *Pb* exposure ends up in the bones and teeth. Children are particularly vulnerable to lead because of its effects on growth and the developing nervous system[17]. Cadmium is mainly absorbed from the lungs, and tobacco smoke is one of the

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Characteristics	N (412)	%
Sociodemographic		
Age as Median (Interquartile range)	32 (20.5)	
Sex-male	212	51.5
Drinking groundwater	238	55.1
Drinking water from rivers	174	44.9
Anthropometric		
Stunting	228	55.3
Wasting	44	10.7
Underweight	140	34.0
Clinical		
Fever in the two last weeks	239	58.0
Diarrhoea in the two last weeks	71	17.2
Cough in the two last weeks	133	32.3
Biochemical		
Anaemia	280	68.0
Inflammation state (elevated CRP)	202	49.0
Iron deficiency	53	12.9
Iron deficiency anaemia	31	7.5
Zinc deficiency	266	64.6
Selenium deficiency	358	86.9
Cooper deficiency	6	1.5

Table 1. General characteristics of the study participants in Popokabaka.

largest single sources of *Cd* exposure in humans[18]. It can accumulate in fatty tissues and human milk and be transferred through breast milk to children. Cadmium toxicity negatively affects reproduction, neurodevelopment, and hepatic, hematological, and immunological systems[10,18]. Mercury is used in agriculture in fungicides or seed preservatives and pharmaceutical catalysts in organic syntheses. Higher levels of *Hg* are often found in seafood, and exposure to this element is also suspected to impair neurodevelopment in children[12] and cause dental, skin, pulmonary, and nephrotic damage[19].

Although the global and nationwide prevalence and burden of HMs are not available, many regional studies[20–26] have estimated the risk of heavy metal exposure in humans. In the Democratic Republic of Congo (DRC), little is known about the risk of heavy metal exposure in children and its spatial distribution. In the non-mining urban region of Kinshasa, Tuakila et al.[27] reported *As* toxicity (95%), *Pb* toxicity (35%), and *Hg* toxicity (10%) in a sample of 100 children in 2014. In the same region, Ngweme et al.[28], in 2021, recently alerted on the toxic detection in leafy marketable vegetables. From the urban mining region of Lubumbashi, Musimwa et al.[29] reported antinomy (*Sb*), *Pb*, and cobalt (*Co)* toxicities in children admitted to a nutrition rehabilitation center. While industrial and anthropogenic pollution can be seen as the most important sources of environmental pollution in many *urban* cities, the heavy metal spectrum from regular non-mining rural communities has not yet been established, including in the DRC. The limitation of these studies is that the spatial distribution and analysis of HMs are not addressed. The spatial aspect is particularly important because it contextualizes HMs to local environmental conditions (water sources, elevation, land cover, and land use), which may affect their distribution. Therefore, combining heavy metal assessment at both the individual and geospatial levels may facilitate understanding the potential geogenic or anthropogenic sources across the region and prepare for ecological health risk intervention patterns. We assessed serum *As, Pb, Hg, and Cd* levels in a community-based representative sample of children under the age of five from Popokabaka, DRC, and searched for relationships with nutrition, health and geospatial characteristics.

Results

Characteristics of the study participants. The study population characteristics have been published previously[30,31]. In short, the median age of the children was 32 months (Table 1). Approximately half (51.5%) of the children were boys, and 55.1% lived within households using groundwater sources for drinking water. Nearly 55% of children were stunted, and more than half (58%) experienced fever within the two preceding weeks of our visit. Approximately one-third (32.2%) reported coughing in the same period. Table 1 also summarizes the burden of essential mineral deficiencies among children in Popokabaka: selenium (Se) deficiency and zinc (Zn) deficiency were highly prevalent in the Popokabaka child population, at 86.9% and 64.6%, respectively.

Table 2 describes the food frequency consumption of children over a recall period of seven days. The results from this table reveal that the regular diet is composed of starchy foods and green leaves. Fish and seafood, known to carry large amounts of minerals from rivers and oceans, were poorly consumed among the children of Popokabaka. Animal source foods such as meat, chicken, eggs, milk, and dairy products were rarely consumed. Sugar and palm oil are frequently used as food additives in this population.

Food groups	Median	p25	p75
Cereals	1	0	5
Vitamin A-rich leaves or tubers	0	0	2
Starch roots & tubers	7	7	7
Green leaves	4	3	5
Vitamin A rich fruits	0	0	1
Other Vegetables	2	1	4
Offal	0	0	0
Meat/chicken	1	0	2
Eggs	0	0	1
Fish and seafood	2	1	4
Vegetable oil	2	1	4
Milk/dairy products	0	0	0
Fatty foods	0	0	1
Sugar/sweeteners products	5	2	7
Coffee, tea, other stimulants	3	0	7
Insects	0	0	2
Palm oil	7	5	7

Table 2. Food consumption characteristics are expressed as the number of days on a week scale the child consumed at least one item of the food groups.

		Detected		
	Undetected <LOD	No quantified LOD-LOQ	Quantified ≥LOQ	Toxic
As	18 (4.4)	148 (35.9)	246 (59.7)	0 (0.0)
Hg	140 (34.0)	238 (57.8)	34 (8.2)	0 (0.0)
Cd	332 (80.6)	76 (18.5)	4 (0.9)	0 (0.0)
Pb	358 (86.9)	38 (9.2)	16 (3.9)	0 (0.0)

Table 3. Prevalence of detection and quantification of heavy metals in Popokabaka Children, n (%) = 412 Children LOD = Limit of detection, LOQ = Limit of Quantification

	Percentiles values Distribution (µg/L)					
	P5	P25	Me	P75	P95	P99
As	<LOD	LOD-LOQ	1.88	5.67	6.12	7.70
Pb	<LOD	<LOD	<LOD	LOD-LOQ	12.8	13.3
Hg	<LOD	<LOD	<LOD	LOD-LOQ	1.5	1.8
Cd	<LOD	<LOD	<LOD	LOD-LOQ	0.09	0.11

Table 4. Percentile value distribution of heavy metals in Popokabaka Children (µg/L). LoD for As 0.2 µg/L, LoQ for As 0.55 µg/L; LoD for Pb 0.6 µg/L, LoQ for Pb 2.1 µg/L; LoD for Hg 0.2 µg/L, LoQ for Hg 0.83 µg/L; LoD for Cd 0.006 µg/L, LoQ for Cd 0.019 µg/L.

Distribution of heavy metals. The results showed that *As, Hg, Cd,* and *Pb* were detected in 95.6%, 66.0%, 19.4%, and 13.1% of the samples, respectively (Table 3). More than half of children (59.7%) had quantifiable arsenic values, while *Hg, Cd,* and *Pb* were only quantified in less than 10% of children without any toxicity level.

Table 4 shows that the distribution was left-censored because of nonquantifiable serum values. Except for *As*, quantifiable values were distributed above the 95th percentile for *Hg, Cd,* and *Pb*.

Interaction between the detection of heavy metals and other nutrition outcomes. The non-parametric Kruskal Wallis test[32] identified two statistically significant relationships (Table 5): first, *As* detection was negatively associated with *Zn* levels. Higher *As* levels were found in Zn-deficient children. Second, *Hg* detection was positively associated with Se levels in Popokabaka children. Children with higher *Hg* levels also had higher *Se* levels. No differences were found concerning anthropometry and growth across HM levels.

	Freq	HAZ	WHZ	WAZ	Se (µg/L)	Zn (µg/dL)	Cu (µg/dL)	Hb (g/dL)
As_bin								
<LOD	18	−2.1 (2.7)	−0.2 (1.2)	−1.3 (1.4)	49.5 (19.0)	65.1 (9.9)	140.5 (55.5)	10.1 (2.8)
[LOD-LOQ]	148	−2.4 (2.1)	−0.5 (1.1)	−1.6 (1.8)	53.7 (22.0)	64.1 (20.1)	146.0 (49.0)	10.3 (1.8)
≥LOQ	246	−2.1 (1.8)	−0.5 (1.4)	−1.5 (1.4)	52.7 (18.9)	59.9 (15.8)	145.5 (47.0)	10.4 (1.7)
P value		0.49	0.87	0.57	0.30	0.002*	0.37	0.94
Hg_bin								
<LOD	140	−2.4 (2.3)	−0.5 (1.6)	−1.7 (1.6)	48.7 (17.0)	60.6 (16.5)	135.5 (49.5)	10.5 (1.5)
[LOD–LOQ]	238	−2.1 (1.9)	−0.5 (1.4)	−1.5 (1.6)	53.5 (18.0)	61.9 (18.5)	148.5 (40.0)	10.2 (1.9)
≥LOQ	34	−2.2 (1.3)	−0.6 (1.6)	−1.5 (0.9)	67.3 (16.0)	64.0 (14.5)	162.5 (57.0)	10.5 (1.5)
P value		0.18	0.83	0.35	<0.001*	0.25	0.046*	0.34
Pb_bin								
<LOD	358	−2.1 (2.1)	−0.5 (1.5)	−1.5 (1.7)	52.7 (20.0)	61.6 (16.6)	145.0 (46.0)	10.4 (1.9)
[LOD-LOQ]	38	−2.3 (1.6)	−0.6 (1.9)	−1.8 (1.7)	53.9 (13.6)	65.9 (19.6)	154.0 (53.0)	10.3 (1.9)
≥LOQ	16	−2.1 (1.7)	−0.2 (1.2)	−1.4 (1.3)	48.9 (18.0)	63.7 (19.5)	137.5 (51.0)	10.5 (1.6)
P value		0.81	0.54	0.46	0.52	0.42	0.55	0.83
Cd_bin								
<LOD	332	−2.1 (2.1)	−0.5 (1.0)	−1.5 (1.6)	52.6 (20.8)	62.2 (17.0)	147.0 (45.0)	10.4 (1.9)
[LOD-LOQ]	76	−2.4 (2.0)	−0.7 (1.3)	−1.7 (1.5)	54.3 (14.5)	59.4 (18.3)	136.5 (41.0)	10.3 (1.6)
≥LOQ	4	−1.9 (1.7)	−0.6 (0.7)	−1.3 (1.3)	48.4 (42.2)	66.4 (23.1)	130.5 (105)	10.3 (0.3)
P value		0.42	0.42	0.17	0.69	0.88	0.13	0.98

Table 5. Differences in the Median (interquantile range) level of some nutrition indicators across the HM levels using the Kruskal wallis test. * means significant pvalue. HAZ = height-for-age Zscore; WHZ = weight-for-height Zscore; WAZ = weight-for-age Zscore; Se = selenium; Zn = Zinc; Cu = copper; Hb = haemoglobin.

Spatial variation in As/Zn and Hg/Se interactions. The spatial distribution maps of *As, Zn, Se,* and *Hg* are presented in Fig. 1. The spatial pattern indicates a low Zn concentration in the northern part of the study region, where *As* is more concentrated. In addition, the spatial distribution of *Se* and *Hg* is almost the same, with a high concentration around the Kwango River. The spatial distribution (Fig. 1), using the global bivariate spatial association index, showed a significant spatial dependency between Hg and Se (L = 0.12, *P* value < 0.001), implying that high concentrations of Hg were spatially associated with high concentrations of Se. A significant spatial discrepancy was observed between *As* and *Zn* (L = − 0.07, *P* value < 0.001). The local bivariate spatial association is presented in Fig. 2, indicating the local contribution of observation to the spatial association. The blue color for the Se–Hg association indicates households where a high concentration of *Se* is significantly associated with high concentrations of Hg. In contrast, for the *As-Zn* association, the same color indicated areas where high values of *Zn* were significantly associated with low values of *As*, thus suggesting a lack of local association.

Discussion

In the present community-based study of children of Popokabaka, we screened for the existence and distribution of the four most significant potential HMs[33]. Arsenic was detected in almost all children (95.6%), of which more than half (59.7%) had quantifiable values. Mercury was detected in 66.0% of children, with fewer (8.2%) having quantified values. Lead and cadmium had relatively low detection rates of 13.1% and 19.4%, respectively. Arsenic was negatively related to Zn, while mercury was positively related to selenium levels, both statistically and geographically. These findings suggest environmental exposures.

Since the arsenicosis crisis report from Bangladesh[34] due to *As* contamination in drinking water, there has been an increasing public health interest in this metal. Recently, two papers[35,36] reported that in Africa, *As* is spatially abundant in water, soil, sediment, fish, and vegetation and advocated for human exposure and health effect assessment. Similarly, our results suggest that *As* is widespread in Popokabaka, and communities could be exposed to a permanent source. For example, as in any rural context, untreated contaminated water used for drinking, cooking, and irrigating crops may be an important source. Consumption of contaminated seafood could also result in elevated *As* concentrations. There is compelling evidence that consumption of predatory fish such as shellfish, sea mammals, and other (shark, swordfish, mackerel, tilefish from the ocean) increase the As level[37]. However, as shown in Table 2, fish and seafood consumption in Popokabaka is relatively low. The geographical inaccessibility of this area also excludes any imported sources of such foods. Arsenic exists in different forms in nature: toxic in acute and poisoning conditions (arsenate, arsenite), nontoxic when metabolized by the body (monomethyl-arsine, dimethyl-arsine), and nontoxic in food (arsenobetaine, arsenocholine). Inorganic *As* is the most lethal and carcinogenic[37]. In this study, we reported the total As concentration and additional analysis is required to specify the form.

Geospatial analysis showed that the highest exposure to *As* in children north of Popokabaka and on the eastern side of the Kwango River. This also implies that the presence of *As* varies in the soil of Popokabaka. In addition, the arsenic was more commonly found in children with more profound Zn deficiency. This interaction

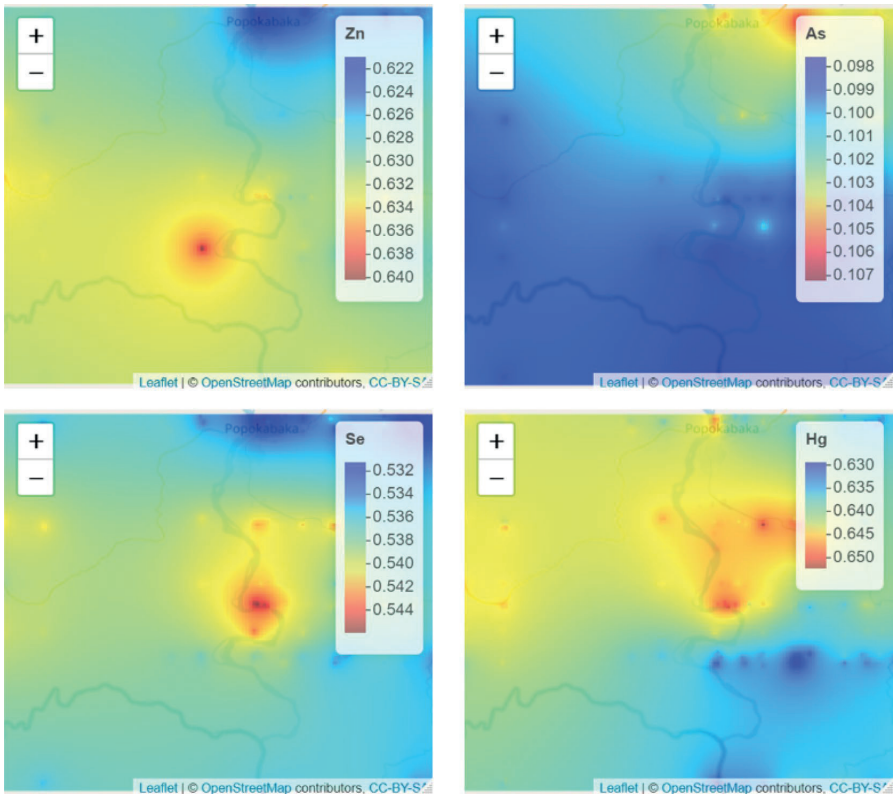

Figure 1. Spatial distribution and variation in As, Zn, Se, and Hg in Popokabaka. For plotting purposes, the concentrations of Se, As, and Hg were multiplied by 10, 100 and 1000, respectively. The inverse distance's weighting is based on the optimum power obtained after the cross-validation.

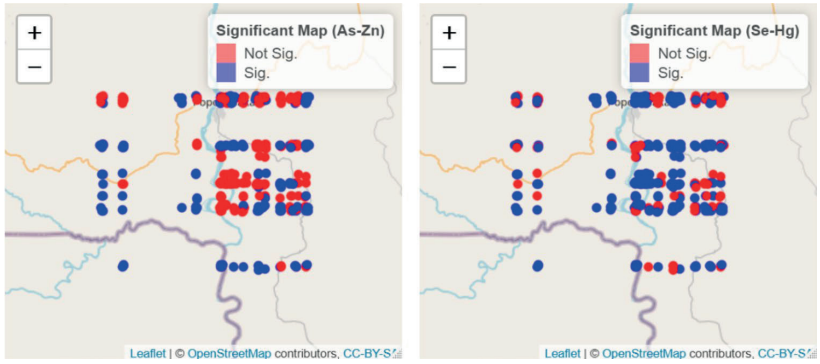

Figure 2. Local bivariate spatial association between As and Zn, and Se and Hg. Blue dots indicate a significant spatial association, and red dots indicate a nonsignificant spatial association. Geographic coordinates were systematically moved to a given direction (200 m) to avoid identifying specific households. This displacement did not affect the spatial relation of points.

between As and Zn is supported in the literature. Kader et al.[38] who reported that presence of the two minerals in the soil could lead to chelation. The authors concluded that Zn uptake in plants was significantly reduced in As-containing soils.

Lead, Hg and Cd usually share the same geogenic or industrial sources and have been more frequently reported from mining or rural regions[24,26–28,39,40]. They leach from geogenic granite rock or industrial pollution and enter the food chain by contamination. They are well-known polluants metal from the environment[33]. Contrarily to arsenic, they are highly toxic, unnecessary for human metabolism, and cause, at a low level, severe damage to the nervous system, development, and behavioral performance[8]. Our data has revealed that the 95th percentiles of Pb, Hg, and Cd were respectively at 12.8 µg/L, 1.5 µg/L, and 0.09 µg/L. No toxic level has been quantified among children, but the proportion detected was quite significant, as 66% for Hg, 19.4% for Cd, and 13.1% for Pb. This high proportion of detection of low Hg levels is of concern and should be a prioritized research question. The literature supports that a diet favoring seafood is associated with a high level of blood Hg[41]. Fish consumption can explain most of the blood Hg in Popokabaka. Even if overall fish consumption is low in Popokabaka, it may likely be higher along the river than in other areas. Also, fish consumption can explain the positive association (statistical and geographic) between mercury and selenium. Both elements are common in fish, and the area of high detection/exposure is along the river. This environmental source and others should be of priority interest in further exploration.

The literature supports inverse relationships between these three metals and children's IQ and growth. Our study found no relationship between the growth and detection of HMs. The Lancet[42] pointed out that co-exposure to multiple metals increases neurotoxicity and leads to a decline in neurocognitive development during early life.

We assessed the four HMs in blood under fasting conditions (> 8 h from the last meals). Another study[43] reported that these four metals have blood concentrations elevated only for a short time after ingestion (4–6 h). They are rapidly metabolized by the liver, accumulate in specific tissues (keratins), and are excreted by the kidney. Considering that, the HM levels we reported could be underestimated[43]. In addition, It was also impossible to capture the chronic exposure and accumulation history. Studies that use matrices such as nails, hair, and urine could complement and improve our understanding. This study revealed a high proportion of value < LoD or < LoQ. These censored data do not simply mean zero value but are undetected with the highly sensitive ICP-MS we have used. In this context, further environmental exploration to assess the risk of exposures for this category. The selection of a spatial interpolation method may impact the distribution map of HMs and their associated minerals. Sophisticated spatial interpolation methods such as kriging could have been applied because they provide the best linear unbiased estimates and highlight local variations[44]. However, it was very complex to fit the semi-variogram, probably due to the distribution of sample points (rand cluster). In some circumstances, the inverse distance seemed to suit this study and its outcomes kriging[44]. In addition, the result of the bivariate spatial association could also have been affected by the number of neighborhoods, which was set to four (standard) in this study.

Despite these limitations, we have reported a picture of heavy metals in a representative rural community for the first time in DRC. Most of the studies are hospital-based and low-scale. The lab analysis method used in this study, ICP–MS, is the most accurate and is indicated in the study of trace elements. Statistical analysis was confirmed and completed by advanced geospatial techniques to better describe the distribution and variability of these HMs.

Conclusions

The high occurrence of As and other HM detection reported in this study implies that Popokabaka should be considered an area with certain HM hazards. Based on existing data, we suggest a geogenic source (soil) and the ingestion of contaminated food and drinking water as possible pathways. However, biomonitoring and deeper exploration are needed to establish environmental causal pathways that could help adapt defensive measures to prevent health and nutrition damage in communities.

Methods

Study design and location. We conducted a community-based cross-sectional study in the Popokabaka Health zone, Kwango Province, DRC, between May and June 2019. The region (5°3,803,500–5°430,000 latitude South, 16°3,406,000–16°370,800 longitude East) is entirely rural without any known mining history (see Fig. 3). It is close to the Kahemba region and the Angola country border, two areas known for diamond mining exploitation. The Kwango River that crosses the Popokabaka region takes its source and crosses these mining regions. Agriculture, which is not varied, has cassava as the principal plant grown. The use of fertilizers is not common among farmers. Konzo, a neurotoxic motor disease, is also prevalent in the region[45–48]. People drink untreated water from diverse groundwater sources. Families live under a house built on earth materials. Growth retardation is severe: one in two children is stunted[49,50]. Information on congenital malformation and cancer is not available.

Participants and sampling. As the present research is part of a multiple-outcomes biomarker survey, the minimum sample size was based on a proportional calculation for anemia prevalence of 0.59, a precision of 0.075, a design effect of two, and a response rate of 0.80. The total size of 412 children aged 12–59 months was considered in this study. Children were selected using a three-stage cluster sampling technique. More details on sampling are described in our former article[31]. They belonged to 5 clusters known as health areas which were selected as part of the cluster sampling procedure. Those were the Kabangu, Ingasi, Cite-Popo, Secteur-Popo, and Tzunza health areas.

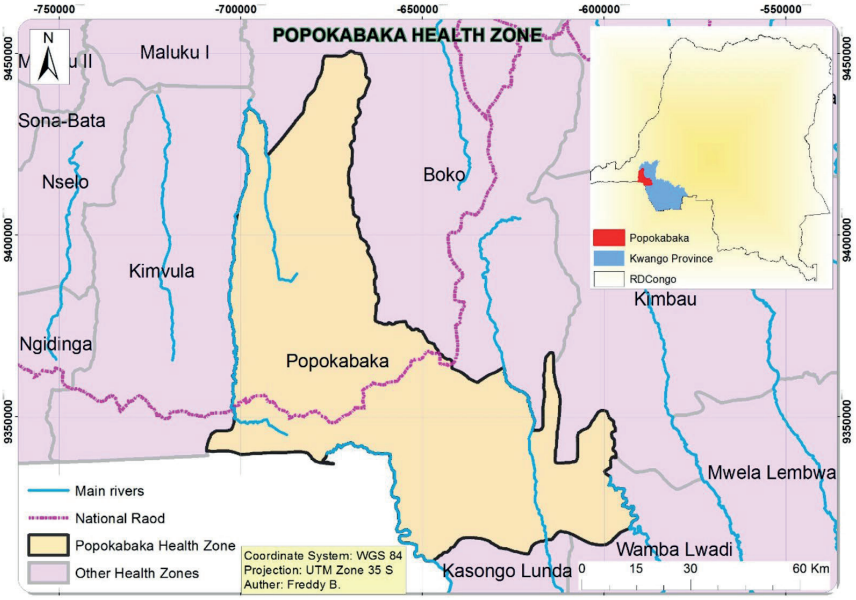

Figure 3. Location of Popokabaka Health Zone. Source: shapefile downloaded from the Humaniterian Data Exchange (https://data.humdata.org/dataset/drc-health-data) and map created by Freddy Bangelesa using ArcGIS 10.4, 2022".

Data collection and blood processing in the field. Data were collected using a questionnaire completed on android tablets using the Survey CTO application. The digital questionnaire consisted of eight modules: household characteristics; water, hygiene, and sanitation (WASH); household food security (Household Food Insecurity Access Scale-HFIAS); child health history; Infant Feeding practices; anthropometric measures; dietary patterns (24 h recall and food frequency); and biochemical sampling. Household geocoordinates (longitude, latitude, altitude, and precision) were captured for children's location using the "geopoint" command in the digital questionnaire. Data collection was organized on two consecutive days in each cluster: a household survey with anthropometry on the first day and blood collection on the following day. More details can be found in our previous papers[30,31].

Blood processing and management. We used serum BD vacutainers (BD-368380), trace-element-free equipment, powder-free sterile disposable gloves, plastic surfaces, and techniques previously described[31] to ensure that samples were not contaminated. We collected up to 6 mL of venous blood from each child and separated the serum from the blood cells within 3 h[30,31]. Separation was performed at 2300 rpm for 10 min (RCF 1532 g) using a Hettich centrifuge (Tuttlingen, Germany). Serum was aliquoted into two 2-ml polypropylene vials (Sarstedt, Nümbrecht, Germany). Vials were stored at −40 °C while completing the fieldwork and then transported from the Popokabaka area to Kinshasa (12 h of vehicle trip) and stored in liquid nitrogen. There, vials were stored at −80 °C at the Kinshasa School of Public Health for a week before being shipped with dry ice to the Norwegian University of Life Sciences' Laboratory (Aas, Norway) for analysis.

Laboratory processing and assessment. *Sample preparation.* Using a 100- to 1000-µL pipette (Sartorius, Göttingen, Germany) and Thermo Scientific ART Barrier pipette tips (Waltham, MA, USA), 250-µL aliquots of thawed, tempered, and homogenized serum were transferred into 5-mL polypropylene tubes (Sarstedt, Nümbrecht, Germany) and accurately weighed (Sartorius MC 210P). Subsequently, using a 10–300 µL electronic pipette (Biohit, Helsinki, Finland), 100 µL of internal standard (rhodium (Rh) and selenium (^{74}Se)) and 500 µL nitric acid (HNO_3, 69% weight (w)/w, sub-boiled ultra-pure) were added to each sample before digestion for three hours at 90 °C in a heating cabinet (Termaks, Bergen, Norge). Furthermore, the samples were quantitatively transferred into polypropylene centrifuge tubes (Sarstedt, Nümbrecht, Germany) and finally diluted to 500 mL with deionized water (> 18 MΩ). To stabilize mercury in the solution, 100 µL hydrochloric acid (HCl, 37% w/w, sub-boiled ultra-pure) was added to each sample.

Analysis of samples. Quantification of the total element concentrations in serum was conducted by inductively coupled plasma–mass spectrometry using the Agilent 8900 Triple Quadrupole (QQQ) ICP–MS[51]. The masses were (Q1/Q2): *Pb* (sum of 206/206, 207/207, and 208/208) with gas mode ammonia (NH_3), *Cd* (111/111), and

Hg (202/202) using gas mode oxygen (O_2). Detection limits (LoD) and quantification limits (LoQ) were standard deviations of the blank samples (n = 10) multiplied by three and ten, respectively. Blank samples were taken through the measurement procedure, including the sample preparation steps. The LoD/LoQ ratios (n = 10) were determined in mg/L as (0.0002/0.00055), *Cd* (0.000006/0.000019), *Hg* (0.0002/0.00083) and *Pb* (0.0006/0.0021).

Quality control. We assessed blank samples for contamination of reagents and equipment used. Accuracy was checked by concurrent analysis of Seronorm™ TEs Serum L1 and L2 (Billingstad, Norway). The obtained data for Hg were within a 95% confidence level of the certified values issued. Compared with analytical values issued, bias equal to 1.5% (L1) and 1.7% (L2) for Cd and 5.0% (L1) and 9.4% (L2) for Hg was noticed, while with respect to Pb in Serum L1 and L2, the results were < LoQ. Despite low levels of As (< LoQ), compared with the analytical values issued, a bias equal to 22% (L1) and 21% (L2) was revealed. The method's within-laboratory reproducibility (RSD) was 12% for *Cd,* while As, *Pb,* and *Hg* were inconclusive since obtained values were below LoQ; the results were obtained by carrying out measurements on 12 replicate samples aliquoted from a pooled sample of serum and measured on three different days. Method's repeatability (RSD) determined on Serum L1 (n = 5) and L2 (n = 5), were 13% and 8.6% with respect to Hg and 11% and 4.0% with respect to Cd. Repeatability of Pb determined by analysis of Serum L1 and L2 were inconclusive. However, regarding As, the repeatability was estimated to 16% (L1) and 17% (L2) calculated on results < LoQ. It is important to note that As was quantitatively determined in 59.6% of the 412 serum samples (Table 3). The repeatability for As is expected to improve for actual serum samples since the measurement uncertainty increases near the LoQ.

Heavy metal thresholds. The upper limits of concentrations of *As* (< 20 µgL-1), *Pb* (< 50 µgL-1), *Hg* (< 50 µgL-1), and *Cd* (< 50 µgL-1) for any acute exposure in children were taken from Carl Burtis and David Bruns[37].

Other nutrition outcomes definition. Anthropometric indices included weight-for-height, height-for-age, weight-for-age, and mid-upper-arm-circumference-for-age, and their Z scores were calculated using WHO Anthro software[52].

Wasting was defined as a weight-for-height Z-score (WHZ) < − 2, stunting was defined as a height-for-age Z-score < − 2, and underweight was defined as a weight-for-age Z-score < − 2. Biochemical measures included Hgb, Cu, Zn, and Se. Anemia was defined as levels < 11 g/dL[14], Iron Deficiency (ID) as Transferrin saturation is < 20%. Cu deficiency as Cu < 80 µg/dL, Zn deficiency as < 65 µg/dL[27], and Se deficiency as < 7.0 µg/dL[10].

Statistical analysis. Data were analyzed using Stata 16.1 (StataCorp LLC, Texas, USA). First, we described the proportions and patterns of data below LoDs, between LoDs and LoQs, and above LoQs cut-offs. Parametric measures such as *means* could not be computed due to the high proportion (> 65%) of undetected/unquantified values (left-censored distribution)[53]. In this case, substitution by unique values and imputation model techniques might overestimate, bias, or fabricate data[54]. Instead, statistics were summarized using a non-parametric method suggested by Tekindal et al.[55] and percentiles (5th, 50th, 75th, 95th, and 99th percentiles). The difference in continuous nutritional outcomes was checked across HMs category levels using the non-parametric Kruskal Wallis test[32]. A significant difference was indicated by $P < 0.05$.

Spatial analysis. Spatial analysis was conducted in R (R Core Team, 2014), and maps were produced using the package leaflet. The spatial analysis concerned only heavy metals with a proven statistical association with essential minerals. This concerns As, Hg, Cu, and Se. We used the inverse distance weighted spatial interpolation approach to map the distribution of these minerals/heavy metals[44,56]. Spatial statistics were applied using Lee's L bivariate spatial autocorrelation test[57] to capture the spatial association between heavy metals and other essential minerals. This test integrates information from Pearson's r (spatial bivariate association measure) and Moran's I (univariate spatial association measure). The weight matrix was defined using the k nearest neighbor approach, and the number of k was set to four[58]. The value of the global L index varies between −1 and 1. Both positive and negative values indicate spatial association—spatial dependency for positive values and spatial discrepancy for negative ones. A value of zero indicates that both variables are randomly distributed (no spatial association). A pseudo-significant test based on Monte Carlo simulation of a stochastic permutation process[39] with 10 000 permutations was computed to measure the significance of the association. The test compares the observed pattern to the theoretical random pattern. The null hypothesis is that there is no spatial association, and the alternative is that there is a spatial association. We set the significant difference at $P < 0.05$. We further computed the local bivariate spatial association to assess the individual area's contribution to the global L and the spatial bivariate heterogeneity.

Ethical considerations. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Norwegian Institutional Review Board" REK. Committee (ref: 2018/1420/R.E.K. vest, date: 30.11.2018) and the Kinshasa School of Public Health ethical committee (ref: ESP/CE/2019, date: 28.01. 2019) and in Bergen. Other authorizations were requested from the local administrative and health authorities. Written informed consent was obtained from mothers or caretakers of children in this study. A systematic spatial displacement of 200 mm was applied to a given direction to avoid identifying concerned households.

Data availability

The dataset of this study can be made available on a reasonable request to BKM.

Received: 22 May 2022; Accepted: 29 October 2022
Published online: 03 November 2022

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Acknowledgements

The Norwegian Agency for Development (NORAD) and cooperation funded this research through the Norwegian Higher Education (NORHED) growing partnership for higher education and research in nutrition (GrowNut Project: QZA-0484, COG-13/0002) in the DRC, and by the collaboration between the University of Kinshasa, University of Kwazulu-Natal, and University of Bergen.

Author contributions

B.K.M., M.A.M., T.A.S. and I.M.S.E. designed the research; B.K.M., E.L.F.G. and M.M.L developed the standard operating procedures development; B.K.M., M.A.M. and I.M.S.E. supervised data collection; E.L.F.G. and M.M.L. conducted the laboratory analysis and prepared Fig. 1; B.K.M., M.M.B., and F.B. performed statistical analysis; F.B. performed geospatial analysis; B.K.M. prepared the original draft; all authors reviewed the manuscript and read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

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Paper IV

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Determinants of Micronutrient-Rich Food Consumption in the Rural Context of Popokabaka, the Democratic Republic of Congo: A Cross-Sectional Study

Journal:	*Public Health Nutrition*
Manuscript ID	Draft
Manuscript Type:	Research Paper
Subject Category:	6. Nutritional epidemiology
Keywords:	Micronutrient, Food, Consumption, Children, Popokabaka

Abstract:	Abstract Objective: Foods, including fruits, vegetables, legumes, fish, meat, dairy, eggs, and insects, are excellent sources of different micronutrients (vitamins and minerals). Unbalanced consumption of such foods may expose the most vulnerable groups to single or multiple micronutrient deficiencies. We aimed to assess food consumption patterns and determinants of the consumption frequency of micronutrient-rich foods among children under five years of age in Popokabaka, Democratic Republic of Congo (DRC). Design: A Cross-sectional, three-stage probabilistic sampling technique. Data were gathered using a validated 16-item Food Frequency Questionnaire (FFQ), the Household Food Insecurity Access Score (HFIAS) questionnaire, the wealth index score based on household ownership, and other individual characteristics. Descriptive statistics and negative binomial regression models were performed. Setting: Popokabaka Health Zone, Kwango Province, DRC. Participants: 432 children and their mothers participated. Results: Green leaves were the most commonly consumed food (92%) used as a source of micronutrients in Popokabaka. Animal sources were consumed to a limited extent (insects, 10%; milk and dairy, 21%; and meat and chicken, 65%). Exactly 88.4% of Popokabaka households

	experienced severe food insecurity access, while 40.7% of children had poor food consumption. Household food insecurity had a negative impact on the consumption of animal micronutrient-rich foods, whereas the wealth index and livestock activity were positively associated with consumption. Conclusions: The present study highlights the importance of diet diversification and household food security, as well as the role of agricultural improvements as a potential contributor to household diet quality and micronutrient adequacy in children of Popokabaka.

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Abstract

***Objective*:** Foods, including fruits, vegetables, legumes, fish, meat, dairy, eggs, and insects, are excellent sources of different micronutrients (vitamins and minerals). Unbalanced consumption of such foods may expose the most vulnerable groups to single or multiple micronutrient deficiencies. We aimed to assess food consumption patterns and determinants of the consumption frequency of micronutrient-rich foods among children under five years of age in Popokabaka, Democratic Republic of Congo (DRC).

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***Conclusions*:** The present study highlights the importance of diet diversification and household food security, as well as the role of agricultural improvements as a potential contributor to household diet quality and micronutrient adequacy in children of Popokabaka.

Keywords: Micronutrient; Food; Consumption; Children

Introduction

Micronutrients, also known as vitamins and minerals, are crucial for healthy growth and cognitive development, particularly in early childhood, when needs increase [1,2]. They play critical roles in multiple biological processes in the body, serving as modulators of various metabolic pathways and as cofactors in immune and antioxidant stress responses. Foods such as fruits, vegetables, legumes, fish, meat, dairy, eggs, and insects are reputed as micronutrient-rich and bioavailable sources in many settings worldwide[3,4]. However, their consumption varies according to geography, the environment, economics, and culture[5]. In developing countries, diets fail to provide adequate micronutrient quality, explaining the persistence of a high prevalence of micronutrient deficiencies (MND) over time[6]. In a recent Lancet Global Health Series, Stevens *et al.*[7] estimated that in 2022, over half (56%) of preschool-aged children worldwide would have MND, with three-quarters living in low- and middle-income countries.

The lack of one or more vitamins or minerals is critical for children, and MND lead to high morbidity, faltering growth, disability, and mortality [6,8]. Zinc deficiency is associated with diarrhea and acute respiratory infections, contributing substantially to child mortality in developing countries[9,10]. Selenium deficiency is linked to the reduced production of glutathione peroxidase, a selenium-dependent enzyme known as a major antioxidant in the immune response. Vitamin A deficiency, the leading cause of blindness worldwide, impairs immune function and cell differentiation and is associated with high morbidity and mortality during childhood[11]. Iron deficiency leads to microcytic anemia, impaired immune and endocrine functions, impaired cognitive function, and decreased capacity to work in adults[12].

Micronutrient deficiencies coexist[13] and may persist if specific and targeted interventions are not initiated[14]. Among all known global nutrition strategies, dietary diversity through frequent consumption of adequate quantities and varieties of micronutrient-rich foods remains the most natural and sustainable solution for preventing and reducing the burden of MND in populations[15-18]. In the Democratic Republic of Congo, data on micronutrient consumption are limited. Moumin *et al.*[19], using repeated 24 h recalls on nonconsecutive days, reported a high prevalence of inadequate iron, zinc, and vitamin A intake in children in the southern Kivu and Kongo central provinces. Although various methods[7,20,21] for summarizing and presenting food consumption data have been utilized in different studies, few have addressed the determinants of frequency.

Popokabaka is a rural setting in the Democratic Republic of Congo (DRC) that offers a wide variety of fresh micronutrient-rich foods, in contrast to the high prevalence of undernutrition in all forms[22]. Recently, in Popokabaka[23,24] we identified deficiencies of essential minerals such as zinc and selenium, but less iron deficiency. Micronutrient deficiencies are preventable[13], and Popokabaka

communities need to be informed about factors that could help them tackle MND. In the present study, we aimed to assess food consumption patterns and investigate the determinants of the consumption frequency of each of these micronutrient-rich food groups in the rural areas of Popokabaka, DRC.

Methods

Survey Design and Setting

The present study was part of a cross-sectional study on micronutrients and diets conducted in May 2019 in the Popokabaka Heath Zone, DRC. The country is one of the most affected by undernutrition worldwide, with a high global hunger index, child stunting rate of 43%, child anemia rate of 59.8%, and under-five mortality rate of 9.9%[25,26]. Dietary diversity is also low, in contrast to huge crop and livestock opportunities[22,27]. Popokabaka is an entirely rural region located at 5°22049.2600 S – 5°2504800 S, 16°20026.1600 E– 16°22014.8800 E. People in this part of the country culturally rely on cassava as a staple food. Konzo, a neurotoxic motor disease, is prevalent in this region and mainly affects children and women (15–18). Access to agricultural fertilizers is limited. Breeding and poultry are practiced less because of the lack of drugs to fight animal epidemics. People avoid fishing because of fear of crocodiles in the Kwango River. Accessibility to imported food and goods from other towns is limited because of poor roads.

Participants and Sampling

A three-stage probabilistic sampling scheme was used to select a representative sample of 432 households with at least one child under five years. Five clusters (health areas) were selected in the first stage using a probability-proportion-to-size technique among the nine possible areas. These were Kabangu, Ingasi, Cite-Popo, Secteur-Popo, and Tzunza. In the second stage, three villages are randomly selected from each cluster. In the last stage, an equal number of 30 households with at least one child aged 12–59 months were systematically selected from a detailed list pre-established by community workers in each village. The main respondent was the mother or primary caregiver. Children for whom the respondent did not provide consent to participate were excluded. More details on the sampling calculations and techniques are provided in our previous publication[23].

Data Collection and Measurement Tools

The study questionnaire encompassed three sections: household sociodemographic information, the Household Food Insecurity Access Scale (HFIAS), child characteristics information, and a validated 16-Food Groups Frequency Consumption questionnaire (FFQ). Sociodemographic characteristics included

mother's age, household size, ownership of various assets, toilet and drinking water types, stored food, and subsistence activity. Using the Food Access Insecurity Scale (HFIAS), mothers were asked nine frequency-occurrence questions about coping strategies for food insecurity over a recall period of four weeks (30 days) for the selected children. The scale measured three domains: anxiety and uncertainty about the household food supply, insufficient quality (including variety and preferences for the type of food), and insufficient food intake and its physical consequences. The FFQ captured the consumption frequency of 16 food groups over a recall period of seven preceding days. The present study assumed that children eat what is in the household (family meals). We computed the Food Consumption Score (FCS), the most commonly used indicator of dietary diversity and nutrient intake[28], and classified it into one of three categories: poor, borderline, or acceptable food consumption[29]. We also calculated a simplified wealth index score variable using principal component analysis of asset ownership, toilet type, and drinking water[30].

Outcomes and Covariates

Four food groups from the 16 FFQ were considered micronutrient-rich food groups and were used as dependent variables (green leaves, meat/chicken, fish/seafood, and insects). These were discrete count variables, defined as the number of days any item in this food group was consumed during the previous seven days. All other household characteristics were considered independent variables in the four regression models.

Statistical Management

Data were collected using structured questionnaires through face-to-face interviews with trained field workers, using the Survey-CTO application and Android tablets. The completed forms were uploaded instantly to the cloud server. The database was downloaded, managed securely, and analyzed using Stata 16.1 (StataCorp LLC, Texas, USA) software at the end of the study. Age and household size are reported as medians (min-max). All categorical variables are described as frequencies and percentages. Because of the discrete nature and overdispersion of dependent variables, we used a negative binomial regression in each of the four models used to search for determinant between covariates and each of four discrete dependent variables (frequency of consumption determined as the number of days per last week of green leaves, meat/chicken, fish/seafood, and insects). Bivariable and multivariable regression analyses were performed, and adjusted regression coefficients were reported with 95% confidence intervals (CIs). All the covariables were entered into the model. Multicollinearity was assessed for all the regression models.

Results

Sociodemographic Characteristics

The sociodemographic characteristics of the households are presented in Table A1. The median age of the women who responded to the survey was 32 years (16-55). Over 50% of the households had more than six persons in their households. Farming was the most common subsistence activity practiced by 92.1% of households, while livestock and fishing were practiced by 49.3% and 29.2% of the households, respectively. Food insecurity was highly prevalent;88.4% of households were severely food insecure, while only 1.6% were food secure.

Food Consumption According to the Food Composition Score

Figure A1 shows the proportion of children who consumed at least one item in the 16 food groups in the last week. From this figure, the "starch and roots-tuber" appeared to be the most popular food group consumed by 100% of children, while the "cereals" was eaten in 65% of children according to the 7-day recall. Milk dairy products, insects, and eggs were the least frequently eaten food groups, identified in 9%, 10%, and 26% of children, respectively.

Focusing on the four micronutrient-rich food groups as previously defined in this study, "green leaves" and vegetables" were also frequently consumed by 97% and 83% of households, respectively, according to the 7-day recall. However, animal-source micronutrient-rich food groups were associated with diet quality in Popokabaka, and the analysis revealed that 40.7% of households had poor food consumption, on average. This proportion increased in Kabangu (44.4%) and Tzunza (59.7%), which are distant health areas from the center of Popokabaka (Cite-Popo, 34.4%; Secteur-Popo, 30.0%). Figure A2 reveals that the proportion of children with adequate food consumption was relatively low (18.7% on average), decreasing from the center to a distant location.

Consumption Frequency of Micronutrient-Rich Foods

The consumption frequency is reported in Table A2, as the number of days a food group was consumed on a weekly recall scale. The "starch roots or tuber" food group was frequently consumed and preferred to the "cereals" group. More than 50% of the children consumed starch every day, while having only cereals one day a week. Table A2 also shows that vegetable micronutrient food sources ("green leaves" and "other vegetables") were more frequently consumed than animal sources ("meat and chicken" or "insects").

Determinants of Consumption Frequency of Micronutrient-Rich Foods

As described in the methods section, four food groups were considered as response variables in the present study. These include green leaves, meats/chicken, fish/seafood, and insects. The same independent variables were checked as determinants of the consumption frequency in each of the four food group models.

HFIAS negatively determined the consumption frequency of meat/chicken and fish/seafood food groups. This indicates that animal micronutrient sources are related to household food security. The higher the household food insecurity, the less frequent the consumption of micronutrient-rich foods. The wealth index score positively determines the frequency of consumption of meat/chicken and insect food groups. This means that an animal's source of micronutrient food is related to its wealth. Wealthier households had more frequent consumption of these food groups than did poorer households. Livestock as a subsistence activity also showed a positive relationship with the consumption frequency of fish/seafood but no relationship with the meat/chicken food group. Engaging in farming activities showed no relationship with any food group. There was also a positive association between the mother's age and the frequency of green leaf consumption. Except for the mother's age, almost all independent variables did not determine "green leaves" consumption in Popokabaka.

Discussion

Frequent intake of micronutrients, including vitamins and minerals, is a diet quality parameter that characterizes good nutrition and health. Communities living in rural settings should have access to diverse and fresh micronutrient-rich foods that prevent the development of MND. The present study assessed food consumption patterns in rural areas of Popokabaka, DR Congo, and investigated the determinants of the consumption frequency of micronutrient-rich foods.

Food Consumption Pattern- HFIAS and FCS

The results reveal that Popokabaka households experience a high prevalence of severe food insecurity-access (89%) based on the HFIAS and a high prevalence of poor food consumption (40.7%) based on the FCS, which is much higher than the national value (15.6 7%) reported in the DRC's Multi-Indicator Clustered Survey MICS 2017[25], implying that Popokabaka inhabitants have a poor nutrient-content diet compared to the national average Congolese population. As stated in the Methods section, the FCS is a validated proxy indicator that can capture nutrient adequacy and diet quality[28], whereas the HFIAS measures the capacity to access food [21]. Literature [31] supports the correlation between the two measures. In Popokabaka, the burden of poor food consumption is accompanied by a household food insecurity-

190 access burden. This differs from the Nyangasa *et al.*[32] study in rural Zanzibari, which reported a high
191 prevalence of poor food consumption (65%) and modest prevalence of severe food insecurity (32%).
192 Food assistance in the emergency Zanzibar context may explain this finding. Under these conditions,
193 households had access to food but had not yet diversified their intake.
194
195 ***Consumption Frequency of Nutrient-Rich Foods***
196 In general, food consumption in Popokabaka is plant based. People rely on plant-source foods, such as
197 green leaves and starchy cassava. Animal-sourced foods are rarely consumed. Meat, fish, insects, milk,
198 dairy products, and egg consumption were uncommon in Popokabaka. A study conducted by Ecker *et*
199 *al.*[33] in three rural settings (Rwanda, Uganda, and Tanzania) in Eastern Africa reported similar results,
200 where the diets were poorly balanced and predominantly plant-based, and animal products were rarely
201 consumed. In a review of global food consumption, Allen[34] also reported that diets in LMICs are
202 primarily plant-based and thus have poor-quality protein and low micronutrient content. Although there
203 is supportive literature[29] of the benefits of a plant-based diet reputed to provide plenty of vitamins,
204 inhabitants with limited animal-source foods intake are, to some extent, at risk of MND They need to
205 add minerals from other sources.
206 Among the surveyed households, crop farming (92.1%) was the main occupation and subsistence
207 activity for Popokabaka's inhabitants. Livestock and fishing are practiced less. The literature[35] supports
208 the notion that rural communities that rely on forest food usually keep demanding animal food sources.
209 Enahoro *et al.*[36] reported that an increase in livestock sector production up to 2025 would increase the
210 intake of micronutrients among all communities.
211
212 ***Determinants of Consumption Frequency of Micronutrient-Rich Foods***
213 In the present study, we considered four groups of micronutrient-rich foods. According to the results,
214 green leaves are the predominant source of the most frequently consumed micronutrient-rich foods.
215 Meat/chicken, fish/seafood, and insects were consumed less. Food security access, wealth, and livestock
216 ownership positively determine the intake of meat, chicken, fish, seafood, and insects. We did not find
217 any determinants for green leaves. Similarly, Fadare *et al.*[21] reported that owning livestock and having
218 good knowledge of micronutrient-rich foods determined micronutrient-rich food consumption. For
219 Speedy[37], wealth appears to be the primary determinant of per capita meat consumption. Based on some
220 studies[38-40], the food insecurity contribution may be explained by the household's low income, which is
221 intended for a wide proportion of production for sale to deal with social problems. A 2019 study in South

Africa [40] reported that households with a livestock component had a lower prevalence of severe food insecurity. We did not observe any statistical collinearity between the two measures in our study.

Strengths and Limitations

The strength of this study is its rigorous methodology. We designed a community-based survey by using a multistage probabilistic method for household sampling. The two indicators used to estimate food consumption patterns were validated. In addition, for the first time, count response variables (and negative binomial regression) were used instead of aggregated binary response variables (logistic regression) to identify contributors to food consumption frequency efficiently. This provided us with a more accurate interpretation of the determinants we found. Collapsing or simplifying these quantitative variables into a binary variable (dichotomization) would lead to the loss of valuable information, power decrease, and statistical inefficiency[41,42]. However, our study has some limitations. Food consumption depends on food availability and production, which varies with seasonality. The consumption patterns reported in this study may differ between the rainy and dry seasons. Our results should be restricted and applicable only during the data collection period (the dry season). Second, at the household level, we assumed that all household members shared the same family dishes. It was impossible to estimate individual nutrient intake under these conditions, especially for those at risk for MND (children, pregnant women, and lactating women). In addition, the number of days per week disregarded multiple intakes within a day or quantity and quality of the foods. Finally, most indicators used were based on recall memory, with an increased possibility of recall bias. To minimize this bias effect, data collectors were trained to ask the mothers to remember the exact food item they had prepared each day of the previous week before giving the frequency of each of the 16 items.

Conclusions

Popokabaka communities experience severe food insecurity and poor food consumption, which explain their poor diet quality and underconsumption of micronutrient-rich foods. The results of the present study support the development of sensitive nutrition interventions, such as livestock activities, which would contribute to the fight against MND. Research is needed to explore food accessibility and production at the market and farm levels.

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Appendix

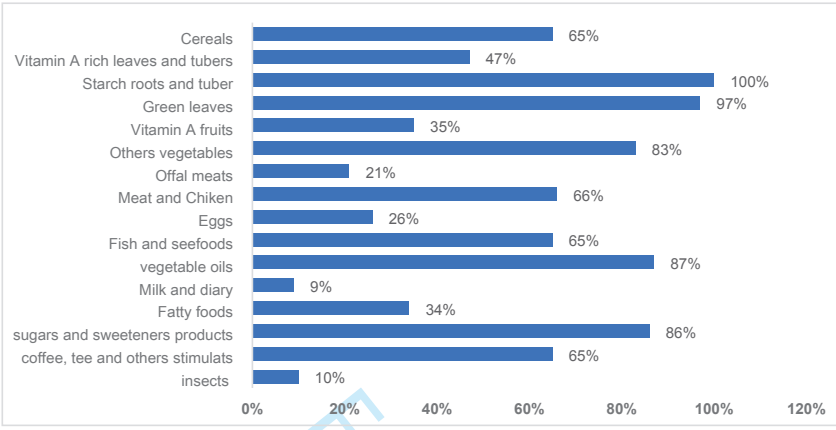

Fig. A1 Proportion of households that have consumed at least one item of the 16 food groups in the last week.

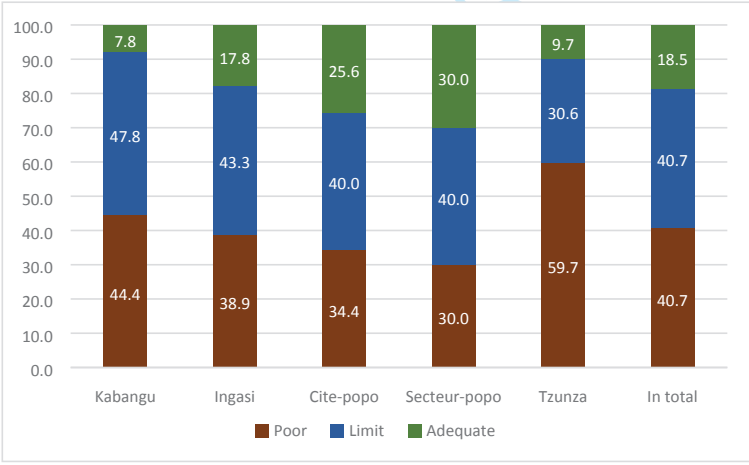

Fig. A2 Distribution of Food Consumption Score (FCS) categories in Popokabaka

359 **Table A1.** Sociodemographic Characteristics of households in Popokabkaba, 2019

		N (%)
Mother's age (years)	*Med (min-max)*	32 (16-55)
Household size	*Med (min-max)*	6 (2-21)
Farming as subsistence	*N (%)*	398 (92.1)
Livestock as subsistence	*N (%)*	213 (49.3)
Fishing as subsistence	*N (%)*	126 (29.2)
HFIAS group	*N (%)*	
Food secure		7 (1.6)
Mildly food insecure		9 (2.1)
Moderately food insecure		34 (7.9)
Severely food insecure		382 (88.4)

360

361 **Table A2.** Frequency consumption of 7-day recall, representing the number of Days a food group was
362 consumed in the last seven days

Food groups	Median	p25	p75
Cereals	1	0	5
Vitamin A-rich leaves or tubers	0	0	2
Starch roots & tubers	7	7	7
Green leaves	4	3	5
Vitamin A-rich fruits	0	0	1
Other Vegetables	2	1	4
Offal	0	0	0
Meat/chicken	1	0	2
Eggs	0	0	1
Fish and Seafood	2	1	4
Vegetable oil	2	1	4
Milk/dairy products	0	0	0
Fatty foods	0	0	1
Sugar/sweeteners products	5	2	7
Coffee, tea, alcohol/other stimulants	3	0	7
Insects	0	0	0
Palm oil	7	5	7

363 p25: the 25th percentile, p75: the 75th percentile,
364

365 **Table A3.** Negative binomial regression model identifying factors associated with consumption
366 frequency of micronutrient foods (number of days consumption). Each cell contains the adjusted β
367 coefficient, with in brackets its 95% CI of z test

	Green leaves	Meat and chicken	Fish and see food	Insects
Mother age	0.009 (0.002; 0.017)	-0.002 (-0.017; 0.014)	-0.007 (-0.018; 0.005)	0.017 (-0.043; 0.043)
Household size	-0.016 (-0.039; 0.007)	0.042 (-0.002; 0.086)	-0.009 (-0.044; 0.024)	0.053 (-0.043; 0.232)
Having farming activity	0.187 (-0.016; 0.390)	-0.106 (-0.484; 0.273)	-0.075 (-0.362; 0.210)	-0.316 (-1.692; 1.060)
Having livestock	0.025 (-0.075; 0.124)	0.164 (-0.041; 0.370)	0.186 (0.033; 0.341)	-0.434 (-1.202; 0.334)
Having fishing activity	-0.052 (-0.160; 0.057)	-0.054 (-0.276; 0.168)	0.044 (-0.119; 0.208)	-0.698 (-1.607; 0.211)
Wealth score	0.013 (-0.012; 0.038)	0.087 (0.038; 0.136)	0.030 (-0.008; 0.069)	0.332 (0.116; 0.547)
HFIAS* score	-0.008 (-0.016; 0.001)	-0.027 (-0.043; -0.011)	-0.016 (-0.029; -0.005)	-0.021 (-0.083; 0.040)

368 * Household Food insecurity access Score

Paper V

Mbunga *et al. Agriculture & Food Security* (2023) 12:36
https://doi.org/10.1186/s40066-023-00443-3

Agriculture & Food Security

RESEARCH

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An example of a convergent mixed-methods analysis to examine food security: the case of Popokabaka in the Democratic Republic of Congo

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Abstract

Background Food insecurity is alarming in all four dimensions—availability, access, utilization, and stability—in Popokabaka, DR Congo. In such cases, a unique indicator may not help to develop adapted and local long-term actions. A comprehensive analysis of food insecurity is needed. We aimed to examine the burden and extent of food insecurity and suggest integrative pathways using a mixed approach for transformative actions at the local level.

Methods We designed a convergent parallel mixed-methods study with four-level data sources collected in Popokabaka: (1) *a household food survey* (using the Household Food Insecurity Access Scale (HFIAS), a Household Dietary Diversity Score (HDDS) and the Food Consumption Score (FCS), (2) *a market food census* (assessing food availability and cost per 100 g), and (3) *an exit food market survey* (assessing buyers' food choices and client satisfaction), and (4) *on-farm qualitative study among food producers* (exploring challenges and opportunities). Descriptive statistics from our quantitative data were triangulated with themes emerging from qualitative data.

Results Popokabaka experienced severe food access insecurity (89%), poor food consumption (40.7%), and low dietary diversity (30.2%) at the household level. The quantitative findings at the household level were linked to market characteristics and farmer-reported themes under three pathways: *poor diet quality*, *culturally grounded diet*, and *risk perception*.

Conclusions The focus should be on improving livestock development, developing adapted communications about nutrition to change established dietary habits, and engaging the government and all stakeholders to empower local communities for improved food security.

Keywords Food insecurity, Mixed methods, Market, Household, On-farm

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Introduction

The progress toward Sustainable Development Goal 2 (SDG 2), which aims to ensure that all people all the time have physical, social, and economic access to sufficient, safe, and nutritious food and aims at eradicating all forms of malnutrition, has stagnated globally (1). According to the 2022 Global Report on Food Crises (GRFC) [2], global hunger levels remain alarmingly high due to a confluence of crises such as COVID-19, climate change, and conflicts [3] that continue to worsen malnutrition among the most vulnerable groups (people in developing countries, rural settings, and poorer households, as well as children) [4]. The 2022 State of Food Security and Nutrition in the World Report (SOFI) [5] estimated that worldwide, between 702 and 828 million people were affected by hunger, an increase of 150 million since 2019. In 2021, approximately 2.3 billion people were moderately or severely food insecure, representing 11.7% of the global population [5]. Concurrently, the 2021 Global Nutrition Report [6] estimated that 149.2 million children under 5 years of age were stunted, and 45.4 million were wasted in 2021.

Food security is an established determinant of people's nutrition [7] and encompasses four complex dimensions: availability, access, utilization, and stability [8]. Each dimension has its indicators and estimation methods, often leading to different conclusions and making a common interpretation difficult [9]. Notwithstanding the progress made on new analytic metrics development, there has been, until now, no consensus on a unique or best indicator that could capture all dimensions of food security at the community level [10–13]. The Integrated Food Security Phase Classification (IPC) [14], a multistakeholder initiative, has suggested a combination of consensus-based indicators, which, unfortunately, use a country-level scale that is difficult to implement at the community level. Designing actions and priorities that improve food security at the community level depends on reliable, complete, and meaningful information [15]. Moreover, such actions may also require that different assessment approaches are mixed to include quantitative and qualitative aspects that incorporate the local context.

Mixed methods allow the harmonization of commonalities and divergences through different analytic methods [16]. They offer researchers and policy-makers a significant advantage in achieving deeper explorations and fostering a comprehensive and linked analysis of the situation resulting from multiple approaches triangulation [16, 17]. Few studies have considered mixed methods in examining food security, and most have considered only one dimension of food security (food access) while not exploring other food chain sites within the same community. Limon et al. [18] in 2017 used a qualitative approach when they considered the household level and mixed the Food Consumption Score (FCS), a quantitative indicator that assesses household access to food, with smallholders' perception. Using an exploratory sequential mixed methods design (QUAL → quant) in the U.S. in 2021, Swindle et al. [19] translated interview themes into survey items to explore the generalizability of their qualitative findings.

The Democratic Republic of Congo (DRC) continues to rank among the poorest countries in the world (175 out of 189 countries based on the 2021 Human Development Index) [20], with one of the world's highest rates of food insecurity [5]. Sporadic sensitive interventions to enhance food security have been implemented across the country. However, decisions must be made and priorities established based on analyzing the situation that integrates all dimensions of food security and different levels of the food chain. However, the burden and extensibility of food insecurity have not been adequately documented when considering each community within its context. Rural states hold a big part of the Food Insecurity burden in DRC [21]. Popokabaka is characterised as a rural Congolese region where food security is expected to be affected in all four dimensions without any war crisis. Thus, it represents a community with common Congolese rural lifestyle challenges. The region also has nutrition evidence [22–28] at the community level to serve local policy decisions.

In the present study, we attempted a parallel convergent mixed analysis to examine food security at different levels of the food chain (household level, market level, and farm level) within the Popokabaka community, DRC, to orient the development and implementation of adapted actions in that area.

Methods

Study design

We designed a parallel convergent mixed-method study [16] that integrates quantitative and qualitative approaches, which were applied at the same time (April–August 2019) within the same study area. Quantitative approaches included assessment of household food access and utilization at the household level (based on a *household survey)*, as well as food availability (based on a *market food survey*) and client satisfaction (based on an *exit survey*) at the market level. The qualitative inquiry explored challenges faced by local food producers through focus group discussions and interviews with key informants. The rationale for mixing the two approaches [29] was to bring together valuable contributions from the household and rural food-market contexts as well as explanations about agriculture production in Popokabaka.

Study site

The study was conducted in the Popokabaka Health Zone, a rural region located in Kwango Province, DRC (Fig. 1). The region extends over 6949 km^2 (5° 22′ 49.26′′ S–5° 22′ 49.26′′ S, 16° 20′ 26.16′′ E–16° 20′ 26.16′′ E) and 200,000 inhabitants, including 38 000 children. The Kwango River, in which many affluent rivers flow, separates the region into two parts. Geographical access is limited due to no paved roads. The community's culture relies on cassava agriculture, as people consume cassava roots and leaves as staple foods. Raising livestock, poultry, and fishing are not practiced as much as cassava growing. There are six official local food markets in Popokabaka, which take place twice a week on regular and set days. Three markets are located on the western side of the Kwango River (Ngasa, Ibuka, and Kisoma), and three are on the eastern side of the river (Citepopo, Imbela, and Kiamfu Kinzadi). Usually, people walk several kilometers to reach the official local market to sell their agricultural production or stock household foods. Several organizations, such as the Food and Agriculture Organization (FAO) [30], CARITAS [31], Congodorpen [32], and ISCO [28], have led projects assisting farmers and providing equipment to support better agricultural production over the last 10 years. However, the situation remains of concern, particularly in some areas with limited road access: Konzo, a food-based neurotoxic motor disease, is prevalent in the region, mainly affecting women and children [26, 27, 33]. Malnutrition and micronutrient deficiencies are also common among children under five [22–24].

Targeted population, sampling techniques, and assessment of variables' criteria

Table 1 summarizes the target study population, sampling techniques, and assessment criteria for each component.

Data collection technique

Quantitative data

All quantitative data were collected through semi-structured interviews using digital questionnaires through the Survey CTO application on an Android tablet; the data were then uploaded to a cloud survey for timely feedback during fieldwork.

– *The household survey*: Based on 432 households, the data gathered included information about household demographics, food stock, Household Food Insecu-

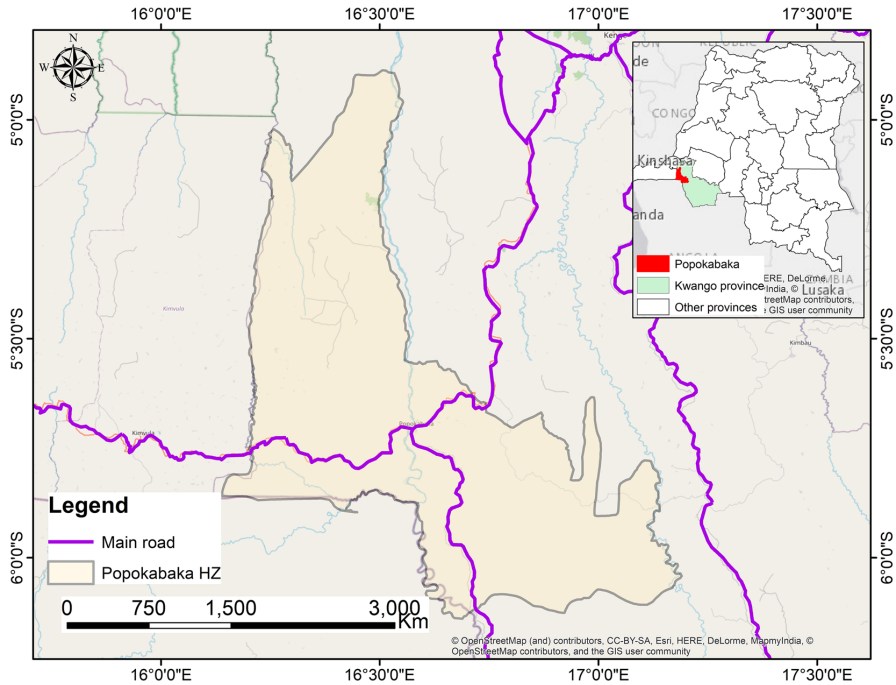

Fig. 1 Location of Popokabaka Health Zone. Source: shapefile downloaded from the Humaniterian Data Exchange (https://data.humdata.org/dataset/drc-health-data) and map created by Freddy Bangelesa using ArcGIS 10.4, 2023

Table 1 Description of target study population, sampling techniques, and criteria judgment

		Study design	Target population	Sampling size and technique	Criteria judgment
A	Household survey	Cross-sectional study	Households with at least one child under the age of five	432 households were selected through a three-stage clustered sampling technique [22], (the main objective of the study was to assess biomarkers)	- HFIAS - FCS - Food stocks
B	Market survey	Food census	Official markets of Popokabaka Food items sold in the markets	Four official markets out of the six markets in Popokabaka (Cite Popo, Imbela, Ngasa, and Ibuka) were randomly selected, including 523 vendors All available food types were recorded. Weight and price were captured	- Food availability - Cost per 1000 g
C	Exit interview survey	Cross-sectional study	Clients that were purchasing food at the local market	147 clients were selected at the exit points of the four official markets of Popokabaka based on a systematic sampling with a sampling interval of 1	- Food choices - Food affordability - Food accessibility - Client satisfaction
D	Focus group discussions	Qualitative case study	Smallholder farmers affiliated with a cooperative farmers' organization possessing crops/livestock	Six FGDs were conducted, including a total of 48 participants purposively selected	Barriers to and opportunities for improved food production in Popokabaka
E	Key informant interviews	Qualitative case study	Community leaders and civil society leaders involved in agricultural community organization	Seven key informants were purposively selected	Barriers to and opportunities for improved food production in Popokabaka

rity Access Score (HFIAS), and Food Consumption Score (FCS).

- *The market survey*: In all four selected official markets, we recorded all foods being sold and interviewed all vendors present on the day of the visit. The food items' name, types of food group, quality, price, volatility, and seasonality were noted. Every food item was weighed using standardized kitchen scales to estimate the cost per 1000 g of net weight.
- *The exit interview*: Information on food choices, accessibility, and satisfaction was collected among 147 clients selected at the markets' exits.

Qualitative data

Qualitative data were gathered using a discussion or interview guide, respectively. Six focus group discussions (FGDs) and seven interviews with key informants (KIIs) were conducted in Lingala language. B.K.M. led all FGDs, and L.E. took notes. All discussions were recorded using a high-quality voice recorder after permission had been obtained from participants.

Data management and analysis

Quantitative data analysis

We reported the frequency and average cost per 1000 g of food within specific food groups. Food items that recurred frequently were pinned and displayed graphically. For each household, three indicators were calculated: the Food Consumption Score (FCS), the Household Food Insecurity Access Score (HFIAS), and the Household Dietary Diversity Score (HDDS) [34]. The FCS classified households as having 'poor' ($FCS \leq 28$), 'borderline' (FCS between 29 and 41), and 'acceptable' ($FCS \geq 42$) consumption. The HFIAS classified households as food secure (HFIAS 1–5), mildly food insecure (HFIAS 6–10), moderately food insecure (HFIAS 11–15), and severely food insecure ($HFIAS \geq 15$). The HDDS was used to classify households as having diversified ($HDDS < 4$) and non-diversified ($HDDS \geq 4$) diets. The analysis was performed in Stata 16.0.

Qualitative data analysis

All digital audio recordings were transcribed verbatim in French. Thematic analysis was used, as recommended by Braun and Clarke [35]. Data reduction and preparation were processed using Atlas.ti 22.0 software. In total, 13 transcripts were read and coded by B.K.M. and L.E independently following two patterns (barriers and opportunities). After data reduction, 114 quotes were identified in the transcripts and clustered around 15 emergent sub-themes for barriers and 13 emergent sub-themes for opportunities. Sub-themes were then grouped into larger themes of barriers and opportunities. Quotations, codes,

and themes were listed in a Microsoft Excel matrix and translated into English at this stage. Finally, the themes were discussed with the remaining coauthors of this study to ensure conformability [36].

Integrating the results through a convergence integration framework

Both quantitative and qualitative information were linked and compared for convergence or divergence during the analysis and interpretation phases of the study using participative discussion techniques with all authors and experts in the food security field.

Results

Food security at the household level

The food security (food consumption and food access) measured at the household level was largely inadequate (see Table 2): the analysis of diet quality through the FCS revealed that only 18.6% of the 432 households visited had adequate food consumption. Moreover, food consumption was poor in 40.7% of the households and borderline in another 40.7% (see Table 2). When assessing households' access to food, the HFIAS revealed a high prevalence of food insecurity in Popokabaka: only 1.6% of households were food secure, while 88.4% were severely food insecure.

Food stock mainly consisted of cassava roots, maize, and palm oil. Farming was the most common subsistence activity practiced in 92.1% of households, while livestock raising and fishing were practiced in 49.3% and 29.2% of households, respectively.

Food availability and cost at the market level

At the four official markets visited, 523 vendors were approached and interviewed. Their median (Interquartile range) age was 32 (21) years, with four women for every man. Among the four markets, the Citepopo Market was the largest, with 232 (44.4%) vendors; it was followed by the Ibuka Market, with 138 vendors (26.4%). In total, 859 food items were recorded, listed, weighted, and evaluated for cost per 1000 g. Table 3 presents the distribution of food type availability across 13 food group categories. Green leaves, oleaginous products, condiments/spices, and manufactured foods were the most available and represented popular food groups sold at Popokabaka's markets. Milk, meat, chicken, eggs, and pulses appeared rare at Popokabaka's markets.

Table 3 Food availability in food groups censed across the four official markets in Popokabaka

	Citepopo	Ngasa	Ibuka	Imbela	Total
Cereals	27	1	4	6	38 (4.4)
Tubers and roots	34	12	20	16	82 (9.5)
Green leaves	48	23	26	5	102 (11.9)
Fruits and other vegetables	44	14	22	11	91 (10.6)
Oleaginous	83	14	23	9	129 (15.0)
Pulses	15	4	3	0	22 (2.6)
Meat	7	5	2	0	14 (1.6)
Chicken/eggs	1	1	5	3	10 (1.2)
Insects	15	2	11	3	31 (3.6)
Fish and sea products	30	5	6	5	46 (5.4)
Milk	8	0	3	8	19 (2.2)
Condiments/spices	110	15	15	21	161 (18.7)
Manufactured foods	42	8	16	48	114 (13.3)
	464	104	156	135	859 (100.0)

Table 2 Food security at the household level determined by the Food Consumption Score (FCS) and the Household Food Insecurity Access Scale (HFIAS) in 432 Popokabaka households

	N (432)	Proportion	CI95%
Food Consumption Score (FCS)			
Adequate	80	18.6	15.1–22.4
Borderline	176	40.7	36.2–45.4
Poor	176	40.7	36.2–45.4
Household Dietary Diversity Score (HDDS)			
Diversified	133	30.8	26.6–35.3
Non-diversified	299	69.2	64.7–73.4
Household Food Insecurity Access Scale (HFIAS)			
Food secure	7	1.6	0.7–3.2
Mildly food insecure	9	2.1	1.0–3.8
Moderately food insecure	34	7.9	5.6–10.7
Severely food insecure	382	88.4	85.1–91.2

Figure 2 displays the most popular food item within each food group available at Popokabaka's markets.

Regardless of the types of food, the most expensive foods were part of fish/sea products, meats, insects, chickens, and milk groups. Table 4 presents the mean food price in Congolese francs (with an estimation in US dollars) per 1000 g.

Food affordability and client satisfaction

A total of 331 clients were selected and interviewed as they were exiting the markets. Most of them were women (with a sex ratio of 3 to 1) with a median (IQR) age of 30 (18) years. In general, more than half of these customers reported that foods were affordable to them. More than 70% testified that they purchased and brought back the food for which they came to the market. Table 5 reports the affordability and client satisfaction in the various markets combined, indicating higher availability than affordability.

Table 6 shows the distribution of food purchased by customers. The 1-day survey reveals that green leaves represented the food most often purchased overall in Popokabaka. However, there were variations depending on which market was under consideration. For example, fish and sea products represented the items most

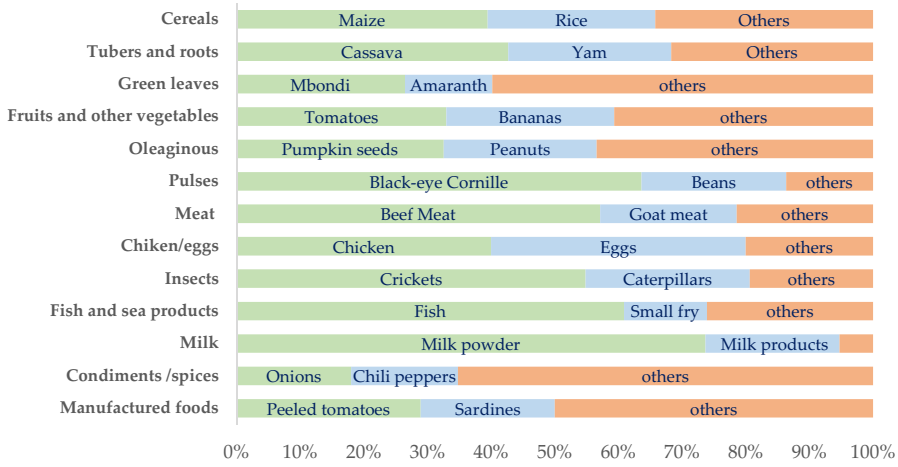

Fig. 2 Most frequent foods items in each food group found in Popokabaka markets

Table 4 Average cost (per 1000 g) of food items recorded at Popokabaka markets

	Average cost with CI $_{95\%}$ in Congolese franc	Average cost + CI $_{95\%}$ in US dollars
Fish and sea products	76,502 (46,108–106,896)	38 (23–53)
Meat	59,744 (13,132–106,348)	30 (7–53)
Insects	41,156 (23,573–58,739)	21 (12–29)
Milk	30,920 (15,293–46,555)	16 (8–23)
Chicken/eggs	27,574 (9480–45,668)	14 (5–23)
Condiments/spices	26,444 (21,438–31,450)	13 (11–16)
Manufactured foods	18,915 (12,941–24,890)	10 (6–12)
Green leaves	12,679 (5881–19,477)	6 (3–10)
Oleaginous	10,118 (7964–12,273)	5 (4–6)
Pulses	8747 (5906–11,589)	4 (3–6)
Fruits and other vegetables	6919 (4791–9048)	4 (2–5)
Cereals	5927 (2289–9566)	3 (1–5)
Tubers and roots	2775 (1829–3722)	1 (1–2)

Table 5 Clients' perceived satisfaction about food availability and affordability when polled at the markets' exits

		Are you satisfied with the food availability?		Total (331)
		No (108)	Yes (223)	
Did you find what you came for?	No	58 (53.7)	27 (12.1)	85 (25.7)
	Yes	50 (46.3)	196 (87.9)	246 (74.3)
Are foods affordable for you?	No	90 (83.3)	18 (8.1)	108 (32.6)
	Yes	18 (16.7)	205 (91.9)	223 (67.4)

Table 6 Distribution of affordability/choice of food items purchased by clients by food groups

	Citepopo	Ngasa	Ibuka	Imbela	Total
Cereals	15	0	1	4	20 (4.5)
Tubers and roots	13	3	3	6	25 (5.6)
Green leaves	45	37	29	2	113 (25.3)
Fruits and other vegetables	8	8	13	9	38 (8.5)
Oleaginous	32	24	8	4	68 (15.2)
Legumes	5	3	1	0	9 (2.0)
Meat	6	8	2	0	16 (3.6)
Chicken/eggs	0	0	1	0	1 (0.2)
Insects	1	0	2	3	6 (1.3)
Fish and sea products	58	11	12	4	85 (19.0)
Milk	0	0	0	3	3 (0.7)
Condiments/spices	21	24	1	1	47 (10.5)
Manufactured foods	9	3	0	4	16 (3.6)
	213	121	73	40	447 (100.0)

often purchased at the Citepopo market, while more fruits were bought at the Imbela market.

Food production

Food production was assessed through a qualitative analysis of barriers and opportunities. Fifty-five individuals participated in focus group discussions and key informant interviews. Twenty-six of the participants were females and they had the following main activities: 21 farmers, eight fish farmers, 23 livestock producers, and three civil society and community leaders. Most of them identified themselves as being affiliated with community corporations. Data analysis identified key themes related to barriers and opportunities to food production in Popokabaka. Additional file 1: Appendix Table S1 presents themes, sub-themes, and a representative quote for each theme.

Barriers included a lack of adequate infrastructure for food production, a lack of motivation in food production activity, a lack of support and control systems, and rudimentary techniques. Overall, the community of Popokabaka relies more on plant cropping activities than livestock and fishing. Despite this, they mostly show a lack of motivation to improve crop production and rely on cassava, maize, and groundnut for home consumption:

> *"We have this will and courage to work and increase our production, but the problem is that we do not get profit from it. Merchants benefit from it a lot. When you sell your products at the merchants' price, you lose a slightly high percentage, 50% of the average revenue you would gain as a retailer. We understand that the road is bad, but this fact also discourages producing in large quantities."*

Participants revealed challenges with livestock production, animal welfare, and lack of veterinarians and vaccines. In addition, it was rudimentary fishing activity, explaining the weak production of animal food sources.

> *"During the 1990s, there was the government's involvement in vaccination campaigns against livestock pests, and there were no tremendous losses compared to what we have now; however, the smallholders today are left to their own fate; they record enormous amounts of losses from their livestock. Although the dry season is known as the period in which livestock pests peak, in Popokabaka, we experience losses at any time of the year. Epidemics that were rare have become routine and permanent. All animals are concerned: pigs, goats, cows, and poultry. We suffer greatly, and this limited livestock provides an animal food source for the community. We truly need help."*

Participants shared statements on Community acceptability, Foods locally produced, Food production activities, and Soil fertility stressing acceptance of items brought to them. They recognized that Popokabaka soil is fertile to any product implying that they will farm any nutritious imported crop with seeds, equipment, and technology provided to them. They also share a high acceptance and desire for new species and varieties introduction for crop and fish farming.

> *"Our soil accepts any crop. Because many of the crops that we use today have been imported, we have tried them, and the soil has responded well. For example, there were not any beans here; we brought them, and people tried them. Today, the soil is responding well. Apart from beans, we also*

brought maize; people tried it, and maize gave them a good yield. There are a few households that use onions, which we brought; the yield grew, and the production was good. This means that the soil in Popocite is very fertile for everything we have brought, even in the old day. There were a few people who grew soybeans, and the yield was good, which means that if a crop is brought to us now, the production will be good because the soil we have is wealthy for everything."

Mixing quantitative and qualitative findings

We suggested three pathways to integrate household indicators with market measurements and farmers' qualitative statements. They were:

1. *The poor diet quality pathway*: linking the high level of poor food consumption in households to low affordability of animal source food at a market level, grounded by a lack of adequate animal production systems and techniques.
2. *The culture-grounded dietary pathway*: linking the low dietary diversity in households to the high availability, at the marketplace, of green leafy foods and culturally grown crops plant, sustained by a lack of motivation to an improved and diversified Agriculture.
3. *The risk perception pathway*: contrasting the food access anxiety in households (expressed by the high level of severe food insecurity in households) with the unexpected satisfaction of buyers in a context of high community acceptability and commitment to their current situation

These pathways are detailed in Table 7.

Discussion and integration

In the present study, different methods and approaches were used to determine food security at different levels of the food chain by integrating information to design an effective action plan. We reported quantitative measures from household and market levels and linked them to qualitative themes we obtained from respondents about food production. Our main results suggested a higher level of poor food consumption, severe food access, and un-diversified dietary practices embedded in factors related to production, technologies, acceptability, affordability and culture. Foods varied at the market level, but animal-source foods were expensive and less affordable. Famers' statements argued for challenges and opportunities that could offer new ways of thinking. In the following paragraphs, we integrate this information into three pathways:

The poor diet quality pathway

Our analysis of food consumption revealed that 40.7% of Popokabaka's households experienced poor food consumption FCS, an indicator used to characterize a community's diet quality, is based on a 7-day food recall, in which the frequency with which foods from various food groups are consumed is weighted differently [37, 38]. Foods from animal sources (e.g., fish/meat and milk/dairy products) are weighted at the highest level, implying that communities that have an inadequate diet are characterized, for example, by a limited consumption of foods from animal sources, and show a high prevalence of poor food consumption as seen in the FCS indicator. When analyzing this pathway, we suspected that the poor quality of diets in Popokabaka would be linked to low consumption of foods from animal sources at the household level, which would explain the fair availability and high cost of these kinds of foods at Popokabaka's local markets. Communities have limited healthier options at relatively higher prices. On the other hand, qualitative interviews indicated that farmers recognized that

Table 7 Food security pathways integrative picture in Popokabaka

Quantitative findings	Qualitative findings	Integrative mixed pathways
Household survey (A) FCS (40.7% of poor food consumption) (B) DD (69.2% of non-diversified diet) (C) HFIAS (88.4% severely food insecure) Market survey (D) fair availability and high-cost animal-source foods (E) high availability of green leafy foods Exit interview (F) affordability of green leafy foods (G) client satisfaction with food purchased	Barriers (1) lack of adequate infrastructure, (2) lack of motivation, (3) lack of support and control systems, (4) rudimentary techniques Opportunities (5) Community acceptability (6) Foods locally produced (7) Food production activities (8) Soil fertility	The poor diet quality (A) ← (D) ← (3) (4) The culture-grounded dietary (B) ← (E) (F) ← (1) (2) (6) (7) The Risk perception (C) ← (G) ← (5) (4)

The arrow ← means 'may be explained by'

livestock and poultry were not developed because of illness. The literature has focused on the link between environmental availability, the accessibility of foods from animal sources, and poor diet quality at the household level. Baltenweck et al. [39] in 2020 reported that local livestock production increases the availability of foods from animal sources, which are better sources of the proteins and micronutrients that are necessary for a healthy population. Hetherington et al. [40] established relationships between livestock ownership, ASF consumption, and nutritional outcomes in children within the same households in rural villages in sub-Saharan Africa.

The culture-grounded dietary pathway

In contrast to the FCS indicator, which provides information about diet quality, the Household Dietary Diversity Score (HDDS) assesses a household's economic capability to access, afford, secure and consume more than four food groups. The score is based on 24 h food recall; when assessed repeatedly, this intake indicates dietary habits and culture. In the present study, diversity in diets was reported as being low (30.8%), meaning that two-thirds of the community did not have a diversified diet. This finding aligns with the availability of the most affordable foods at the market level, the low cost of leafy green vegetables, and the foods that are produced in the greatest quantities and most often mentioned, i.e., cassava, maize, and groundnuts. Previous research [25–27, 33, 41] conducted in the same area reported the prevalence of the traditional monotonous cassava diet and linked it to the prevalence of Konzo disease. We found that culture may influence food production choices, shaping food availability. Culture also influences dietary habits. Even if food is available, preferences, affordability and taboos can limit a household from adopting a diverse diet.

The risk perception pathway

The information generated by the HFIAS indicator is used to assess the prevalence of household food insecurity (based on the access component) and compare changes over time [42]. In contrast to the FCS and HDDS, that indicator is based on 30-day food recall and includes nine questions about the respondents' perceptions of food vulnerability or anxiety and their behavioral responses to that insecurity. Based on this indicator, 88.9% of households were classified as severely food insecure. This result implies that a considerable proportion of households include people who are anxious and uncertain about their respective households' food supply, the insufficient quality of the food they have access to, and their insufficient food intake. This perception of risk did not converge with nor diverge from the results of the qualitative inquiry about client satisfaction in food purchasing and community acceptability. It was found that people did not stock up on food at the household level; indeed, they regularly got their food supply on each official market day. As they recall their food intake in the long term, they perceive the risk; however, as long as they could get enough food from what was available, they felt satisfied and developed a kind of positive defiance in the face of severe food insecurity. Some authors have described how the perceived risk of food insecurity and this high level of defiance are used to create local solutions.

Strengths and limitations

Food security is a complex and multifaceted concept, and needs multiple assessment methods to develop actions to address the issues within a community [15]. Mixed methods research is especially powerful to harness the strengths and counterbalance the weaknesses of both qualitative and quantitative approaches [29, 43]. This article complemented specific indicators and mixed information that explain the context of Popokabaka and suggest pathways of food insecurity that need to be addressed to achieve something at the household or individual levels. During this study, each component of the mixed method was assessed following rigorous methodologies to provide reliable findings. In our study the findings were largely convergent which supports trustworthiness of the qualitative findings. At the last stage of analysis, qualitative and quantitative data were integrated. This mixed approach was feasible and is suggestive of other settings. The interest of using such an approach assessment is recent [17] and continue increasing under food security sector [18, 19].

However, this mixed methods approach also has some limitations. First, the three pathways proposed are suggestive rather than exhaustive or absolute. However, based on our extended stay in the research region and our deep exploration and participatory approach, we suggest that these pathways are relevant to describe food security in the communities under study. Second, foods are highly linked to seasonality. The assessments we made at the market and within households should be repeated during the rainy season to compile an average situation. However, we expect that the food security situation described here is at its worst during the dry season and is probably better during the rainy season.

Conclusion

Regardless of the specific indicator used, the level of food insecurity in Popokabaka is alarming and requires effective actions to avert the most vulnerable individuals from experiencing negative nutritional and health

consequences. The present mixed-method analysis suggests that actions should focus on improving livestock development, developing an adapted communication strategy about nutrition to change engrained dietary habits and empowering the community for improved food security.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40066-023-00443-3.

Additional file 1: Table S1. Themes and supporting quotes describing barriers and opportunities for food production.

Acknowledgements
The authors thank the participants and community members of Popokabaka for their collaboration and participation in this study.

Author contributions
BKM, MAM, IE and TS conceptualized the protocol of this research; BKM, EL, MAM and IE supervised fieldwork and data collection; BKM and EL performed data analysis; BMK prepared the original draft; all authors reviewed and contributed to editing the manuscript. Project administration: MAM, AH, and IE administered the project.

Funding
This research was funded by the Norwegian Agency for Development and Cooperation (NORAD), the Norwegian Higher Education (NORHED) Growing partnership for higher education and research in Nutrition (GrowNut Project: QZA-0484, COG-13/0002) in the Democratic Republic of Congo (DRC), and through the collaboration between the University of Kinshasa (DRC), the University of Kwazulu-Natal (South Africa), and the University of Bergen (Norway).

Availability of data and materials
Data sets, transcripts, and codes matrices served to this article are available as per request to the corresponding author.

Declarations

Ethics approval and consent to participate
The present study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Regional Committee for Medical and Health Research Ethics of Western Norway (ref: 2018/1420/R.E.K. vest, date: 30.11.2018) and the Kinshasa School of Public Health ethical committee (ref: ESP/CE/2019, date: 28.01. 2019). Other authorizations were requested from the local administrative and health authorities before any field work (households, markets, or food production places). Written informed consent was obtained from mothers or other caregivers for the household survey as well as from merchants and clients in the market survey. For the qualitative study, oral informed consent was obtained from participants before starting any discussion. We also obtained permission prior to recording any discussion. Confidentiality was maintained both in data management and when quoting respondents.

Consent for publication
All authors have agreed to the published version of the manuscript.

Competing interests
The authors declare no competing interests.

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Received: 22 March 2023 Accepted: 21 September 2023
Published online: 23 November 2023

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