FACTORS INFLUENCING MICRONUTRIENT STATUS IN SCHOOL CHILDREN AND ROLE OF INDIGENOUS VEGETABLES FOR IMPROVING MICRONUTRIENT INTAKE IN RURAL TANZANIA

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EXTENDED ABSTRACT

Introduction: Deficiencies of iron, zinc and vitamin A in the body continue to affect the health and wellbeing of women and children in Tanzania; consequently, leading to retarded growth during childhood and poor cognitive development, hence, reduced learning capacity and poor school attendance. Moreover, micronutrient deficiencies lower immunity; therefore, reduce ability of the body to fight infections, making children highly susceptible to infections. Factors that contribute to micronutrient deficiencies include inadequate consumption of diverse foods, high prevalence of infectious diseases and inefficient utilization of micronutrients in the body due to persistent inflammations and heavy parasitic infestations. This study aimed at determining the prevalence of micronutrient deficiency and associated factors among school children living in rural households of Kilosa and Chamwino districts in Tanzania. The study determined the concentration of haemoglobin in the blood and status of micronutrients iron, zinc, vitamin A, vitamin E and carotenoids in the serum. The anthropometric measurements of the school children were also assessed to establish their nutritional status. Furthermore, the nutrient intake of school children and the micronutrient composition of selected indigenous leafy vegetables (ILVs) commonly consumed in study areas were also determined to provide data to guide their consumption.

Methods: This study used a follow-up design in which two sequential cross sectional surveys were conducted. The baseline survey was conducted in July-August 2016 to assess the nutritional and micronutrient status of the school children prior to the implementation of an integrated home gardening intervention which started in July 2017- May 2018. The second or endline survey was conducted in July-August 2018 one year after the implementation of an integrated home gardening intervention. The study population included school children of age between five and ten years, who were enrolled to the study

together with their mothers or caregivers. The sample size at baseline was 666 child-mother or caregiver pairs obtained through a simple random sampling technique. The study areas were purposively selected based on the Scale-N project criteria; this included Dodoma region, where Chamwino district was selected and was represented by Mzula and Chinoje villages; for Morogoro region, Kilosa district was selected and was represented by Tindiga and Mhenda-Kitunduweta villages.

Data on socio-demographic variables such as age, gender and morbidity were collected using a pretested questionnaire whereas for dietary intake a 24-hour recall method was used. Anthropometric status was assessed using measurements of weight, height and mid-upper arm circumference (MUAC). Serum concentration of retinol (vitamin A), carotenoids and tocopherols (vitamin E) were determined using the high-performance liquid chromatography (HPLC) while iron status markers (ferritin, soluble transferrin receptor), infection or inflammation markers (C-reactive protein, α-1 glycoprotein) by a sandwich enzyme-linked immune-sorbent assay (ELISA) technique and serum zinc by a spectrophotometric method. School children-mothers or caregivers pairs were followed for two years to assess anthropometric, dietary and biochemical parameters (serum micronutrients and infection markers). In the selected ILVs the concentration of provitamin A carotenoids, tocopherols, and ascorbic acid (AA) were determined using HPLC; the minerals iron, calcium, magnesium, zinc, and phosphorus were determined using Inductively Coupled Plasma-Optical Emission Spectrophotometry (ICP-OES), while phytic acid content was determined using a photometric method.

Results: At baseline the overall prevalence of stunting was 28.1%, underweight 14.4%, and overweight was 5%. Micronutrient deficiencies showed varied prevalence; whereby 43% of the children had anaemia, 29.3% showed deficiency of iron (ID), 24.9 % were

vitamin A deficient (VAD), and 26.4% had zinc deficiency (ZnD). The overall prevalence of reported malaria and diarrhoea was 30.7% and 20.7% respectively. Dietary intake data indicated that, only small proportions of children reached the recommended daily micronutrient intakes for zinc (4%), vitamin A (19%) and B vitamins (14–46%), except for iron (74%). Stunting was highly associated ($p < 0.001$) with underweight in both districts and with VAD in Chamwino ($p < 0.05$). Anaemia was mainly predicted by ID, VAD, and ZnD in Chamwino while in Kilosa it was predicted by elevated infection markers, C - reactive protein (CRP) and α-1 glycoprotein (AGP). Higher serum carotenoids indicative of a diet high in fruits and vegetables was associated with the lower risk of VAD whereas elevated CRP and/or AGP increased the risk of VAD.

The micronutrient content (provitamin A carotenoids, tocopherols, ascorbic acid and minerals which are iron, calcium, magnesium and zinc) of the selected ILVs commonly consumed in the study areas was high. Beta-carotene concentration was high ranging between 2.91 and 4.84 mg/100 g (fresh weight) in ILVs including *Amaranthus spp*, *Sesamum angustifolium* and *Corchorus trilocularis*. This amount could provide more than 50% of the recommended nutrient intake (RNI) for vitamin A. The level of iron was high (34.5–60.4 mg/100 g) in ILVs including *Cleome hirta* and *Sonchus luxurians* and capable of providing more than 50% of RNI for iron. *Amaranthus ssp*. had high levels of calcium, magnesium and zinc and these amounts could provide 85%, 207% and 21% of RNI per 100 g, respectively. *Cleome hirta* and *Cleome gynandra* had high ascorbic acid content more than 15 mg/100 g, and could provide 34 –35% of RNI for ascorbic acid. *Sesamum angustifolium* was the only ILV with high tocopherol content (7.34 mg α-TE/100 g). The highest phytate concentration was found in *Amaranthus ssp*., which could negatively affect its role as a very good source of minerals. After the implementation of an integrated home gardening intervention, the prevalence of anaemia decreased from 42.7’% to 30.6%, and

vitamin A deficiency from 24.5% to 0.4% ($p<0.001$). Consumption of vegetables, fruits and legumes significantly increased from baseline to the end-line survey (87% vs 98%, 63% vs 69% and 76% vs 87%), $p<0.001$, respectively. Moreover, households that reported to grow vegetables increased from 76.6% to 82.1%, ($p<0.05$); awareness on pocket gardening increased from 21.6% to 92.9%, ($p< 0.001$) and proportion of households practicing pocket gardening increased from 3% to 76.4% ($p < 0.001$) from baseline to the end-line survey.

Conclusions: School children in the districts of Chamwino and Kilosa, Tanzania, are simultaneously affected by low energy intake, anaemia, infections such as malaria, micronutrient deficiencies, and inadequate diets. Moreover, significant variations in micronutrient status and dietary habits between districts were observed. Long-term nutritional deficits as reflected by high prevalence of stunting and current micronutrient status, especially vitamin A, iron and zinc, underlines the importance of targeting school children in national nutrition and health surveys for nutrition assessment and surveillance. The analysed indigenous leafy vegetables can potentially make a considerable contribution towards the requirements for nutrients, particularly vitamin A and iron, which are micronutrients of public health significance among school children in the study areas. The significant decrease in the prevalence of anaemia and vitamin A deficiency among the school children during post intervention phase, suggests the potential of integrating nutrition sensitive interventions such as home gardening and nutrition education for better nutritional outcomes. Moreover, programs that reduce infectious diseases and improve hygiene are essential to ensure quality utilization of nutrients in the body.

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LIST OF PUBLICATIONS

i. Gowele, V. F., Kinabo, J., Jumbe, T., Rybak, C. and Stuetz, W. (2021). High Prevalence of Stunting and Anaemia is Associated with Multiple Micronutrient Deficiencies in School Children of Small-Scale Farmers from Chamwino and Kilosa Districts, Tanzania. *Nutrients*, 13 (5):1576.

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DECLARATION

I, Victoria Flavian Gowele, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work done within the period of registration and has not been presented and will not be presented at any other University for a similar or any other Degree award.

Victoria Flavian Gowele **(PhD Candidate)**	**Date**

The above declaration is confirmed by:

Prof. Joyce L. Kinabo **(Supervisor)**	**Date**
Dr. Theresia Jumbe **(Supervisor)**	**Date**
Dr. Wolfgang Stuetz **(Supervisor)**	**Date**

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DEDICATION

This thesis is dedicated to my family. To my beloved husband Dr. Peter Onauphoo Siyao, my dear children Brian, Careen and Caroline and cherished parents, Flavian Isaack Gowele and Margaret Martin Mbago. Thank you so much for your love and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

AGP - α-1 Glycoprotein

AMDR - Acceptable Macronutrient Distribution Ranges

ANOVA - Analysis of Variance

BMIAZ	-	Body Mass Index for Age Z-Score Children
CRP	-	C - reactive protein
DGLV	-	Dark Green Leafy Vegetables
FANTA	-	Food and Nutrition Technical Assistance Project
FAO	-	Food and Agriculture Organization
HAZ	-	Height-for-age z-score
HB	-	Haemoglobin
ID	-	Iron Deficiency
IDA	-	Iron Deficiency Anaemia
IFAD	-	International Fund for Agricultural Development
ILV	-	Indigenous Leafy Vegetables
IoM	-	Institute of Medicine
MAAIF	-	Ministry Of Agriculture, Animal Industry And Fisheries
MoHCDGEC	-	Ministry of Health, Community Development, Gender, Elderly and Children
MUAC	-	Mid-upper-arm circumference
NIMR	-	National Institute for Medical Research
RNI	-	Recommended Nutrient Intake
SF	-	Serum Ferritin
sTfR	-	soluble Transferrin Receptor
STH	-	Soil Transmitted Helminths
TDHS-MIS	-	Tanzania Demographic and Health Survey and Malaria Indicator
UNICEF	-	United Nations Children’s Fund
URT	-	United Republic of Tanzania
VAD	-	Vitamin A Deficiency

WAZ	-	Weight-for-age z-score
WHO	-	World Health Organization
ZnD	-	Zinc Deficiency

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Malnutrition refers to both undernutrition and overnutrition (WHO, 2010). Undernutrition can result in stunting, wasting, underweight or micronutrient deficiencies; over-nutrition is caused by eating more food than the body needs and results in overweight and obesity (WHO, 2010). Hunger and malnutrition remain a challenge worldwide as in 2019, nearly 750 million or almost one in ten people in the world were exposed to severe levels of food insecurity (FAO, IFAD, UNICEF, WFP and WHO 2020). Moreover, nine out of ten stunted children below five years lived in Africa representing 40% of all stunted children in the world (FAO, IFAD, UNICEF, WFP and WHO 2020). According to Best *et al*. (2010) undernutrition for children below five years has been the nutritional priority in low and middle income countries (LMICs). However, school age children and young adolescents in LMICs are also at risk of undernutrition because they are often already stunted, have anaemia, and/or have existing infections from childhood (Lillie *et al*., 2019). A review on nutritional status of school aged children 5-12 years in developing countries and countries in transition, revealed high prevalence of malnutrition in African school-aged children, with 22% suffering from stunting, 7% of overweight, 36% of thinness, 29% of anaemia, 29% of iron, 32% of vitamin A deficiency and 54% of zinc deficiency (Best *et al*., 2010). Malnutrition among school age children in developing countries, especially in Africa, has been linked with diets lacking adequate nutrients (energy, protein, vitamins and minerals) and inefficient utilization of available nutrients due to infections and parasitic infestations (Amare *et al*., 2012; van Stuijvenberg *et al*., 2015).

One of the important vulnerable groups, but often neglected by public health interventions, is school-aged children (Abizari *et al*., 2017). School-age (5–9 years) is a dynamic period of growth and development. Poor nutritional status greatly affects both the cognitive and physical development of children in this age group (Best *et al*., 2010; Tariku *et al*., 2018). Because of late school enrolment in LMICs primary school age also includes early adolescence (10-14 years), a period when skeletal and brain growth spurts and sexual maturation dramatically increase nutrient requirements (Fiorentino, 2015). Malnutrition begins at pre-school period and if left untreated, may progress into school age causing negative effects on the academic performance and general well-being of school children (Getaneh *et al*., 2019). Targeting school children is important because undernutrition in this stage contribute to poor school attendance, poor classroom performance, increased morbidity, and poor general well-being, resulting in poor educational attainment and low intellectual and physical abilities in adulthood (Getaneh *et al*., 2019).

1.2 Nutrition Situation of School Children in Tanzania

In Tanzania, school children above 5 years of age are currently not targeted in national nutrition and health surveys. Therefore, data on nutritional status and a detailed assessment regarding micronutrient intake and status of school children is currently lacking at national level. However, some local studies on the nutritional status of school children reported the existence of all forms of malnutrition (Mwaikambo *et al*., 2015; Nicholaus *et al*., 2020). In rural Tanzania, stunting, anaemia, iron, and vitamin A deficiency are among the most prevalent nutritional problems in school children as studies reported a high prevalence of stunting (21–79%), anaemia (29–80%), and deficiency of iron (33%) and vitamin A (32%), depending on the study area (North-western, Central, or Southern Tanzania) and the age group of the children (Lwambo *et al*., 2000; Tatala *et al*., 2008; Mboera *et al*., 2015; Munisi *et al*., 2016; Kinung'hi *et al*., 2017). In addition, a_{12} study on nutritional status of

school children aged 7-14 years living in urban Tanzania reported 10.2 % and 4.5 % prevalence of overweight and obesity respectively (Mwaikambo *et al*., 2015). These results confirm that malnutrition in Tanzanian school children is prevalent in both urban and rural areas, and micronutrient deficiencies can coexist with increasing overweight and obesity. In addition, the urban new lifestyles increase sedentary activities among school children living in urban areas, and thus, factors such as going to school using private cars or school buses and more time spent on computer or video games are reported to increase the risk of overweight and obesity (Mwaikambo *et al*., 2015).

1.2.1 Acute and chronic malnutrition during school age

Chronic undernutrition results from long-term exposure to food of insufficient quality and quantity, including restricted intake of energy, protein, fat, micronutrients, essential amino acids, vitamins and minerals (Campisi *et al*., 2018). Primary school age is a dynamic period of physical growth as well as of mental development of the child (Elema, 2018; Getaneh, *et al*., 2019). Undernutrition among school children is manifested in the form of stunting and wasting (Getaneh *et al*., 2019). Stunting is the result of chronic or long-term nutritional deprivation due to poor diets, recurrent infections, inadequate maternal or child caring practices, and access to health services and healthy environment (WHO, 2010; Elema, 2018). *S*tunting results in delayed mental development, poor school performance and reduced intellectual capacity (WHO, 2010). A study by Fiorentino (2015) indicated that stunting had adverse consequences at adult age as low women height is associated with small pelvic size, which increases risk of obstetric complications during delivery. Moreover, women of short stature are at greater risk of delivering an infant with low birth weight, contributing to the intergenerational cycle of malnutrition, as infants of low birth weight or retarded intrauterine growth tend be smaller as adults (WHO, 2010).

Wasting or thinness reflects acute or recent nutritional deprivation usually due to inadequate food intake or a high incidence of infectious diseases especially diarrhoea (WHO, 2010; Getaneh *et al*., 2019). Wasting during school-age and adolescence may delay pubertal maturation (Fiorentino, 2015). Furthermore, wasting impairs the functioning of the immune system and can lead to increased severity and duration of and susceptibility to infectious diseases and an increased risk for death (WHO, 2010) .While undernutrition continues to be a concern, with increasing globalization and development, LMICs are also experiencing an increase in overnutrition, creating a "dual burden" of under- and overnutrition (Tzioumis *et al*., 2014). Obese or overweight school children are at a more risk of suffering from type 2 diabetes and cardiovascular diseases (Fiorentino, 2015).

1.2.2 Vitamin A function and deficiency

Vitamin A deficiency is a threat to child health caused by low consumption of vitamin A rich foods and insufficient provitamin A in the diet. Vitamin A deficiency (VAD) not only impairs eyesight but it also prevents the child's body from developing a strong immune system essential for warding off pathogens, hence putting a child at risk of dying from infection (Shekhar, 2013). Vitamin A deficiency is associated with anaemia and stunting, and it is reported to co-exist with iron and zinc deficiencies (Abizari *et al*., 2017; Bationo *et al*., 2018). Scholars like Abizari *et al*. (2017) and Gebremedhin, (2014) reported that supplementation with vitamin A to improve iron status and control anaemia induced by either iron deficiency or infection. Additionally, vitamin A supplementation is reported to combat growth retardation suggesting a potential to effect catch-up growth in undernourished children (Mwanri *et al*., 2000).

Vitamin A is present in a wide range of foods containing either preformed or provitamin A (Tanumihardjo *et al*., 2010). Foods derived from animals including egg yolk, liver and fish provide preformed vitamin A (retinol) that is easily digested and absorbed in the body (Greiner, 2013). Green leafy vegetables, orange fruits, and yellow-colored vegetables are rich in carotenoids (Bationo *et al*., 2018). Some carotenoids are precursors of vitamin A (provitamin A carotenoids) that can be converted in the body into active vitamin A form; while others serve other physiologic purposes for example, lutein in eye health (Mondloch *et al*., 2015). The three major sources of provitamin A carotenoids found in fruits and vegetables are α-carotene in carrots, β-carotene in spinach, and β-cryptoxanthin in citrus fruits and green maize (Tanumihardjo *et al*., 2010). Results from a fully controlled dietary feeding study on men and women in Canada, conclude that serum carotenoids mainly β-cryptoxanthin and lutein can be used as robust biomarkers of fruits and vegetable consumption, and are considered to be reliable biomarkers of dietary carotenoid intake (Couillard *et al*., 2016). Moreover, some studies affirm that serum concentrations can be used to verify specific vegetables or fruit in the diet (Mondloch *et al*., 2015; Bationo *et al*., 2018) . Because of the consequences of VAD on health, increased morbidity and retarded growth, vitamin A intake and status should be monitored among school children.

1.2.3 Iron function and deficiency

Iron deficiency occurs when the intake of total or bioavailable iron is inadequate to meet iron demands, or to compensate for increased losses (Balarajan *et al*., 2011). Iron has an important role in oxygen transportation, as a component of haemoglobin (Fiorentino, 2015). Worldwide, iron deficiency (ID) is the leading cause of anaemia, of which its causes are multifactorial, ranging from micronutrient deficiencies such as iron, folate and vitamin B_{12} to infectious diseases such as malaria and worm infections (Balarajan *et al*., 2011; Desalegn *et al*., 2014). School children are vulnerable to anaemia because of their

higher iron need to meet the demands of puberty and adolescence (Ayogu *et al*., 2015). Interactions between iron and other dietary factors play a significant role in determining the adequacy of iron nutrition. Dietary iron bioavailability is low among populations that consume monotonous plant-based diets with little meat. This is due to the non-haem iron present in plant based diets whose absorption is increased by meat and ascorbic acid, but inhibited by antinutritional factors such as phytates (Sanou *et al*., 2010). An increase in the amounts of meat and citrus fruits, and addition of iron-rich condiments was associated with a decrease in anaemia and iron-deficiency anaemia among Burkinabe children (1–6 year old); moreover, the authors concluded that, meeting iron requirements through diet helped attaining requirements for energy and other nutrients (proteins, zinc, vitamin A, vitamin B_{12}) and deworming was reported to have a positive effect on iron status (Sanou *et al*., 2010). In children, ID is known to affect cognitive performance, increase morbidity from infectious disease, and impair growth (Motadi *et al*., 2015; Abizari *et al*., 2017). Therefore, iron is a key nutrient in the growth and development of school children as ID has implication in anaemia leading to increased morbidity, absenteeism, and indirectly to impaired school achievement in school children (Fiorentino, 2015).

1.2.4 Zinc function and deficiency

Zinc has important role in multiple aspects of metabolism because it forms an integral part of more than 200 enzymes and has significant task in nucleic acid metabolism, cell replication, tissue repair, and growth (Amare *et al*., 2012). Zinc deficiency is associated with stunting and impaired gastrointestinal and immune functions (Uusiku *et al*., 2010; Wessells and Brown, 2012). Foods with the highest zinc concentration include dairy products, red meat, legumes, fortified cereals, and whole grains (Lim *et al*., 2013; Gammoh and Rink, 2017). However, the contribution of plant based foods to zinc intake is uncertain as these foods are low in bioavailable zinc compared to animal based foods

(Gammoh and Rink, 2017). Tanzania is among the countries where vulnerable groups such as growing children are subsisting on plant-based diets, and no national statistics on zinc deficiency is currently available. A study conducted to estimate the global prevalence of zinc deficiency report that over a quarter (>25%) of Tanzanian population has inadequate zinc intake (Wessells and Brown, 2012). Therefore, due to its importance in growth; zinc status is of particular interest in school children as it can affect school performance and reproductive health during puberty, particularly where child-bearing occurs early in life (Schulze *et al*., 2014).

1.2.5 Other micronutrients function and deficiencies

Vitamin A, iron and zinc are the most studied micronutrients as deficiencies are very prevalent around the world, and their adverse effects on health and development on children are well described (Fiorentino, 2015). Nevertheless, vitamin E and B_{12} are among the most limiting micronutrients in the diets of school children (Gowele *et al*., 2021). Vitamin B_{12} is mostly present in animal source foods such as milk, meat, and eggs (Pawlak *et al*., 2014) and it is essential for DNA synthesis, cellular energy production, brain development, and cognitive function whereas inadequate vitamin B_{12} intake or absorption impairs the production of red blood cells (Venkatramanan *et al*., 2016; Leary, 2010). In addition, vitamin B_{12} deficiency has been associated with greater risk of grade repetition and school absenteeism in school children aged 5–12 years (Duong *et al*., 2015). In low- and middle-income countries where the intake of animal-based foods is low (Bationo *et al*., 2018), school children are particularly vulnerable to vitamin B_{12} deficiency.

Vitamin E is a fat-soluble antioxidant and a group of compounds divided into tocopherols (α, β, γ and δ) and tocotrienols (MC Lobo *et al*., 2019). The vitamin E form with the highest biological activity and mostly abundant in the human tissues is α-tocopherol

(Traber, 2014; MC Lobo *et al*., 2019). Vitamin E is involved in the protection of polyunsaturated fatty acids and other cell membrane components against free radical oxidation (MC Lobo *et al*., 2019). In addition, some research suggest a wider biological role for α-tocopherol, including cognitive performance, reproductive and neurological processes by regulating cell division and gene expression (Traber, 2014; Fiorentino, 2015; MC Lobo *et al*., 2019). The major dietary sources of vitamin E are vegetable oils, nuts, whole grains, sunflower seeds and green leafy vegetables (Dror and Allen, 2011; Traber, 2014). Moreover, low α-tocopherol concentrations may result from diets low in vitamin E in combination with inadequate fat, protein, and energy intake (MC Lobo *et al*., 2019). Vitamin E deficiency is rarely found in adults but is more frequently found in children due to limited stores and rapid growth, thus allowing deficiency symptoms to be readily apparent (Traber, 2014). Even though vitamin E is a natural component of various foods, the prevalence of low vitamin E status in school children from both developed and developing countries (33.0% in Brazil and 20.2% in Tunisia) has been reported (Fares *et al*., 2011; do Carmo Custódio *et al*., 2020). Low vitamin E status in children is associated with frequent infections, anaemia due to increased erythrocyte haemolysis, and stunted growth (Traber, 2014; Fiorentino, 2015). In more advanced cases, progressive neurological disorders and muscle deterioration may occur (MC Lobo *et al*., 2019).

1.3 Dietary Patterns of School Children in Tanzania

Despite the wide variety of food produced in Tanzania, the daily diets in most households are very limited in diversity. The report from Comprehensive Food Security and Nutrition assessment of 2017 revealed that 97% of Tanzanian households consumed cereals whereas 17% reported to consume roots, tubers and plantains; in addition, consumption of eggs and meat was reported by only 5% and 17% of households respectively (URT, 2017).

Results from the studies on dietary patterns of Tanzanian school children reported consumption of monotonous cereal-legume meal with low intake of fruits, vegetables and animal source foods (Nicholaus *et al*., 2020) . In addition, consumption of high fat and sugar snacks and beverages that are often energy dense but micronutrient poor was also reported (Mwaikambo *et al*., 2015) . School children spend more time at school, away from their parents or caretaker's guidance and consume more street food compared to younger children. Consequently, changes in food patterns due to the transition from preschool to school age can affect nutrient intakes especially in absence of school feeding programs (Fiorentino, 2015). Best *et al*. (2010) reported that school is an opportune setting for addressing the health and nutrition needs of children. In addition, Nicholaus *et al*. (2020) reported that dietary and other lifestyle behaviours formed during childhood and adolescence can contribute to adulthood behaviours and thus, school is a good platform for reinforcing good dietary practices and health behaviour in the future.

1.3.1 Contribution of indigenous leafy vegetables to micronutrient intake

Indigenous leafy vegetables have been part of the traditional food system and play an important nutritional role in the dietary structure of rural Tanzanians (Gowele *et al*., 2019). Indigenous leafy vegetables (ILVs) are good sources of essential micronutrients such as vitamin A and C, iron, zinc, calcium, and magnesium (Shayanowako *et al*., 2021). In addition, ILVs contain important non-provitamin A carotenoids such as lutein and its isomer, zeaxanthin that are reported to play an important role in supporting visual as well as cognitive function across the lifespan (Stringham *et al*., 2019). Indigenous leafy vegetables are mostly gathered from the wild, with few selected species being cultivated, usually as part of a mixed cropping system in home gardens or smallholder plots (Maseko *et al*., 2018). Common examples of cultivated ILVs include Black night shade *(Solanum nigrum)*, Cowpea leaves (*Vigna unguiculata*), Spider plant (*Cleome species*), amaranth

(Amaranthus species), Sweet potato leaves (*lpomeas species*) and Pumpkin leaves (*Cucurbita species*) (Gowele *et al*., 2019; Adera *et al*., 2021). ILVs are readily available and cheap, and their consumption adds flavour, taste and diversity to the cereal-based staple diets of most rural people (Masarirambi *et al*., 2010; Ejoh *et al*., 2021).

The consumption pattern of ILVs differs among households within different countries. In Tanzania, the consumption pattern is highly variable and depends on factors such as age of consumers, intended benefit, cultural background, poverty status and geographical location (Weinberger and Msuya, 2004; Keding and Yang, 2009; Kimambo *et al*., 2018). Older age individuals use ILVs more with preference attached to their medicinal properties (Kimambo *et al*., 2018). Poor households residing in rural areas use ILVs more than their wealthier counterparts (Weinberger and Msuya, 2004; Keding and Yang, 2009). In Kenya, consumption of indigenous leafy vegetables is limited to higher education levels for urban dwellers (Gido *et al*., 2017). Moreover, despite the potential of ILVs to contain bioactive compounds with beneficial effects on health, they may also contain antinutritional factors (phytates and oxalates) that compromise digestion and absorption of vital nutrients such as iron and zinc (Uusiku *et al*., 2010). Dietary diversity coupled with traditional food preparation and cooking methods (fermentation, boiling, or frying) are reported to be essential strategies to reduce the effects of the antinutritional factors and improve availability of vital nutrients in ILVs (Ilelaboye *et al*., 2013; Patricia *et al*., 2014; Hailu and Addis, 2016; Essack *et al*., 2017). The role of ILVs as sources of micronutrients is even more important given the high prevalence of micronutrient deficiencies of vitamin A, iron, zinc and their associated problems of anaemia and stunting in rural farming communities (Gowele *et al*., 2021). In Tanzania, ILVs contribute significantly to the intake of micronutrients, particularly of resource poor households, where approximately half of vitamin A and one-third of iron requirements are consumed through indigenous leafy

vegetables (Weinberger and Msuya, 2004). Figure 1.1 shows the most common indigenous leafy vegetables that participants reported to consume in the study areas.

Cleome hirta (Mwilile or Mhilile)

Cleome gynandra (Mgange)

Justicia heterocarpa (Mwidu)

Bidens pilosa (Mashona nguo)

Sonchus luxurians (Sunga)

Corchorus trilocularis (Mlenda Mgunda)

Wild Amaranthus spp (Mchicha Bwasi)

Amaranthus spinosa (Mchicha Chawa)

Figure 1.1: Indigenous leafy vegetables commonly consumed in the study areas.

1.4 Biomarkers of Inflammation and Micronutrient Status

In developing countries, infection and undernutrition are prevalent and demonstrate a synergistic relation (Bresnahan and Tanumihardjo, 2014). Infection implies that the body's structure and/or normal metabolism have been interfered with by the entry of material recognized as foreign within the tissues whereas the biochemical and physical changes in a body that are initiated in response to tissue damage or a foreign organism are termed the acute phase response (APR) or inflammatory response (Thurnham and McCabe, 2010).

The systemic acute phase response (APR) leads to an increased production by the liver of a number of plasma proteins which are known collectively as the acute-phase proteins APPs (Jain *et al*., 2011). Acute-phase proteins (APPs) are a class of proteins whose plasma concentrations increase (positive acute-phase proteins) such as C-reactive protein (CRP), a1-acid glycoprotein (AGP), and ferritin or decrease (negative acute-phase proteins) for example retinol-binding protein in response to inflammation (Merrill *et al*., 2017).

Infection and tissue damage can be recognized by their clinical effects on the body but, in apparently healthy people subclinical infection or inflammation can only be recognized by measuring inflammation biomarkers in the blood (Thurnham and McCabe, 2010).

In various settings, populations experience recurrent exposure to inflammatory agents that catalyze fluctuations in the concentrations of acute-phase proteins and measures of micronutrient status, including indicators for vitamin A, zinc, iron, and haemoglobin status (Bresnahan and Tanumihardjo, 2014; Merrill, *et al*., 2017). Therefore, in order to accurately assess micronutrient status in a population, particularly in developing countries where the burden of infection may be high, it is important to measure biomarkers of inflammation as well as of nutrition in prevalence surveys of nutritional status in apparently healthy individuals (Thurnham and McCabe, 2010).

1.5 The Role of Integrated Home Gardening Intervention in Improving Micronutrient Intake and Status

Integrated home garden interventions that combine training in gardening practices with nutrition education has the potential to improve nutrition behaviour, access to vegetables, dietary diversification, supply of essential micronutrients and thus contribute to improve health and nutritional status of resource poor households (Baliki, *et al*., 2019; Blakstad *et al*., 2021). Fruits and vegetables from home gardens are good source of micronutrients especially in the poor households (Suri, 2020). Promoting home gardening, along with awareness of human nutrition is commonly practiced in developing countries to encourage dietary diversity, allowing families to improve their food security, health and nutritional status (Thamilini *et al*., 2019). In such countries, integrated home garden interventions that combine training in gardening practices with education about nutrition knowledge have shown an improved nutrition behaviour (Baliki *et al*., 2019). A recent controlled intervention investigating the impact of an integrated home garden intervention on vegetable production and consumption of rural households in Bangladesh yielded promising results (Baliki *et al*., 2019). Three years after the intervention, the average vegetable production per household increased by 49% compared to baseline (Baliki *et al*.,

2019). In addition, a previous study in Bangladesh reported an increase in the proportion of mothers who consumed dark green leafy vegetables from 37 to 86 percent, and daily vitamin A intake in retinol equivalents (RE) from 30 to 230 in mothers and 10 to 40 in children a year after the implementation of gardening and nutrition education surveillance project (Taher *et al*., 2002).

Results from a 5-year homestead food production programs coupled with nutrition education in Asia (Bangladesh, Cambodia, Nepal and Philippines), revealed improvements in consumption of animal based foods (liver and eggs) and a decrease in anaemia prevalence among children aged 6-59 months in all countries (Talukder *et al*., 2010). Likewise, a decrease in anaemia, wasting, and diarrhoea in children aged 3–12.9 months was reported after implementing an integrated agriculture, nutrition and health behaviour change communication program in Burkina Faso (Olney *et al*., 2015). In Tanzania, a recent controlled study from villages in Pwani region found positive effects in terms of improved consumption of dark green vegetables and peas after 1 year of implementing home gardening intervention (Blakstad *et al*., 2021).

1.6 Justification

Micronutrient deficiency is a global challenge to health and most prevalent in developing countries like Tanzania. Lack of awareness about importance of micronutrients among the targeted beneficiaries has resulted in low intake of micronutrient rich foods hence deficiencies prevail. Micronutrient deficiency can affect any age group, but young children and women of reproductive age are the mostly affected group (Ahmed *et al*., 2012). Childhood is a period of rapid growth, thus during this stage of life micronutrient deficiencies can lead to retarded growth, anaemia, reduced immune function, and impaired motor and cognitive development, all of which may adversely affect academic

performance through reduced learning capacity and poor school attendance as well as longer-lasting effects on productivity and economic potential during adulthood (Osei *et al*., 2010; Moench-Pfanner and Kraemer, 2015).

On the contrary, data on nutrition and health status of school age children is largely lacking in many developing countries including Tanzania, which compromises the targeting of nutrition interventions to this age group (Moench-Pfanner and Kraemer, 2015). In addition, one or a few micronutrients have been evaluated in studies assessing the micronutrient status of school children in most developing countries making it difficult to assess the extent of multiple deficiencies among this age group (Osei *et al*., 2010). This study therefore, aims at assessing the micronutrient and anthropometric status; and their associated factors in school children 5-12 years living in rural areas of Chamwino and Kilosa districts in Tanzania.

1.7 Study Objectives

1.7.1 Overall objective

To assess the micronutrient status and their determinants, micronutrient composition of indigenous leafy vegetables, and effect of integrated home gardening intervention on micronutrient intake and status of school children aged 5-12 years in rural households of Chamwino and Kilosa districts in Tanzania.

1.7.2 Specific objectives

i. To assess the micronutrient status (iron, zinc, vitamins A, E and carotenoids), haemoglobin concentration, anthropometrics and their associated factors among school children in rural households of Chamwino and Kilosa districts in Tanzania.

ii. To determine the nutrient intake of school children in rural households of Chamwino and Kilosa districts in Tanzania.

iii. To determine the micronutrient composition of selected indigenous leafy vegetables commonly consumed in rural households of Chamwino and Kilosa districts in Tanzania.

iv. To examine the effect of integrated home gardening intervention on anthropometrics, haemoglobin, micronutrient intake and status of school children in rural households of Chamwino and Kilosa districts in Tanzania.

1.8 Definition of key concepts

School children: a generic name that refers to children age 5-9 years and adolescents 10-12 years (Fiorentino, 2015).

Adolescence: refers to children falling between the ages of ten and nineteen years, with early adolescence taking place between ten and fourteen years of age and late adolescence occupying years fifteen to nineteen (WHO, 2005).

Household: refers to people who live together, sleep under the same roof and take meals together at least four days a week (Coates *et al*., 2007).

Micronutrients: is the collective term used to describe vitamins and minerals needed by the body in very small amounts. However, their impacts on a body's health are critical, and deficiency in any of them can cause severe and even life-threatening conditions (WHO, 2021).

Anti-nutrients: are chemicals present within the plant to protect the plant as a defense mechanism and aid in other biological functions. They reduce the ability of nutrients such as minerals, vitamins and even proteins within the plant material. This, in turn, affects the nutritional value of these plants (Essack *et al*., 2017)

Bioavailability: is defined as the fraction of nutrient that is absorbed and available for utilization in normal physiological functions or for storage (Tanumihardjo *et al*., 2010).

Anaemia: a condition in which the haemoglobin (Hb) concentration in the blood is lower than normal. It is diagnosed when the concentration of haemoglobin falls below established cut-off values based on age, gender and physiological condition (WHO, 2017).

Nutritional anaemia: result from insufficient bioavailability of nutrients needed to meet the demands of haemoglobin and red blood cells synthesis such as (iron, vitamin B_{12}, and folic acid) and absorption enhancers such as vitamin C (Balarajan *et al*., 2011).

Indigenous leafy vegetable: refers to a crop species or variety genuinely native to a region, or to a crop introduced into a region where over a period of time it has evolved, although the species may not be native (Weinberger, 2004).

Home gardens: refers to a mixed cropping system that encompasses vegetables, fruits, plantation crops, spices, herbs, ornamental and medicinal plants as well as livestock that can serve as a supplementary source of food and income (Galhena *et al*., 2013).

Home gardening: refers to the cultivation of a small portion of land which may be around the household or within walking distance from the family home (Odebode, 2006).

Integrated home garden intervention: refers to interventions that combine training in gardening practices with education about nutrition knowledge (Baliki *et al*., 2019).

1.9 Conceptual Framework of Multiple and Interrelated Factors Influencing Micronutrient Status in School Children

Determinants of micronutrient deficiencies are many, and they have been grouped as underlying and immediate factors while recognizing that they are interlinked. These factors are detailed in Figure 1.2 and in the text as they are regarded as major risks to adequate nutrition in school children. The most immediate cause of micronutrient deficiencies is poor nutrient intake through inadequate diets (Bhandari and Banjara, 2015).

In Tanzania, 97% of households rely mainly on cereals to meet household dietary needs, consumption of micronutrient rich foods such as eggs (5%) and meat (17%) is rather minimal. Similarly, diets are less diversified as 41% of households are able to include up to three food groups in their daily meal (URT, 2017). Diseases and inflammations also, influence the body's ability to absorb and retain micronutrients as in the case of zinc and other minerals loss during diarrhoea. Vitamin and mineral nutrition is severely compromised by infection and diseases such as malaria and worm infestations (Munisi *et al*., 2016). The deficiencies caused by diseases leave the individuals more vulnerable to further illness and less able to absorb micronutrients (Bhandari and Banjara 2015; Munisi *et al*., 2016). The underlying causes of micronutrient deficiencies include insufficient access to food, poor caring practices and inadequate access to health care services, clean water and sanitation (Bhandari and Banjara, 2015).

The limited access to food in adequate quantity and quality is mainly due to poverty and seasonal variation whereas inadequate childcare results from sub-optimal feeding practices and caring capacity (time); further, poor access to healthcare, clean water and sanitation, may lead to increased illness and thus lead to multiple micronutrient deficiencies (MAAIF, 2015). The causes of micronutrient deficiencies are multiple and interconnected as shown in Figure 1.2.

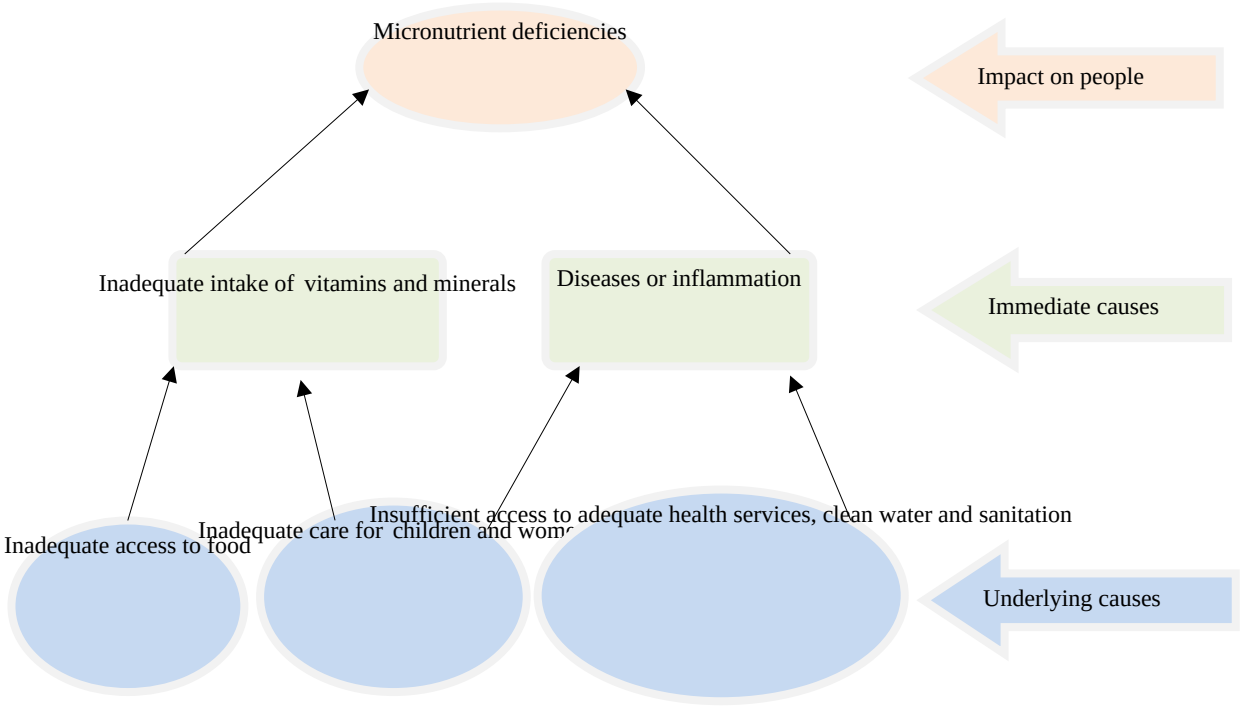

Figure 1. 2: A conceptual framework on interlinkage among causes of micronutrient deficiencies. Modified from UNICEF (1998) and Bhandari and Banjara (2015).

1.10 Methodology

1.10.1 Study design

A follow-up study design was employed in which two sequential cross sectional surveys were conducted. In this study design always the same participants are included when data are collected at different time periods (Keding, 2010). Therefore, data were collected from the same participants in two phases namely; baseline and end-line phase. The baseline data

were collected between July and August 2016, followed by home gardening intervention (October 2017 to May 2018) that was implemented in parallel with nutrition education (September 2017 to April 2018). The individual households were followed up during the intervention phase and the end-line data collected two years after the baseline (July-August 2018).

1.10.2 Study area and population

This study was part of the Scaling-up Nutrition project (Scale-N) implemented in Morogoro and Dodoma regions, Tanzania. Morogoro and Dodoma regions were purposively selected because of their high prevalence of anaemia among children age 6-59 months (66% versus 48%) respectively (TDHS-MIS-2015/2016). One district from each region was further selected, Chamwino district from Dodoma and Kilosa district from Morogoro; further, two villages from each district were purposively selected based on Scale-N project criteria namely; Mzula and Chinoje from Chamwino district and Tindiga and Mhenda-Kitunduweta from Kilosa district.

Kilosa district is located in East central Tanzania characterized with sub-humid climatic condition with short rains starting in October to December and long-term rainfall begin in February to May. This area has flat plains, highlands, and dry alluvial valleys with annual rainfall between 600 –800 mm. Legumes, rice, sorghum, maize and horticultural crops are common in the area, with some livestock integrated into the livelihood system (Graef *et al*., 2017).

Chamwino is located in the central plateau of Tanzania characterized by a dry Savannah and periodically semi-arid type of climate with annual rainfall between 350-500 mm. The district has long dry season starting late April to early December, and a short single wet

season starting December to mid-April. The food system is primarily based on millet and sorghum with deep attachment to livestock (Mnenwa and Maliti, 2010; Graef *et al*., 2017). Other crops commonly cultivated include sunflower, groundnuts and Bambara nuts. The main food sources of vitamin A, iron and zinc in the study areas are indigenous green leafy vegetables such as amaranth (*Amaranthus spp*.), jute mallow (*Corchorus olitorius*); cereal staples such as pearl millet; animal source foods from livestock keeping; legumes and nuts. Fruits such as baobab (*Adansonia digitata*) provide vitamin C mostly in the semi-arid areas of Chamwino district whereas mangoes although seasonal, are a good dietary source of provitamin A carotenoids in both districts. Figure 1.3 shows the study areas that Scaling-up Nutrition project (Scale-N) was implemented.

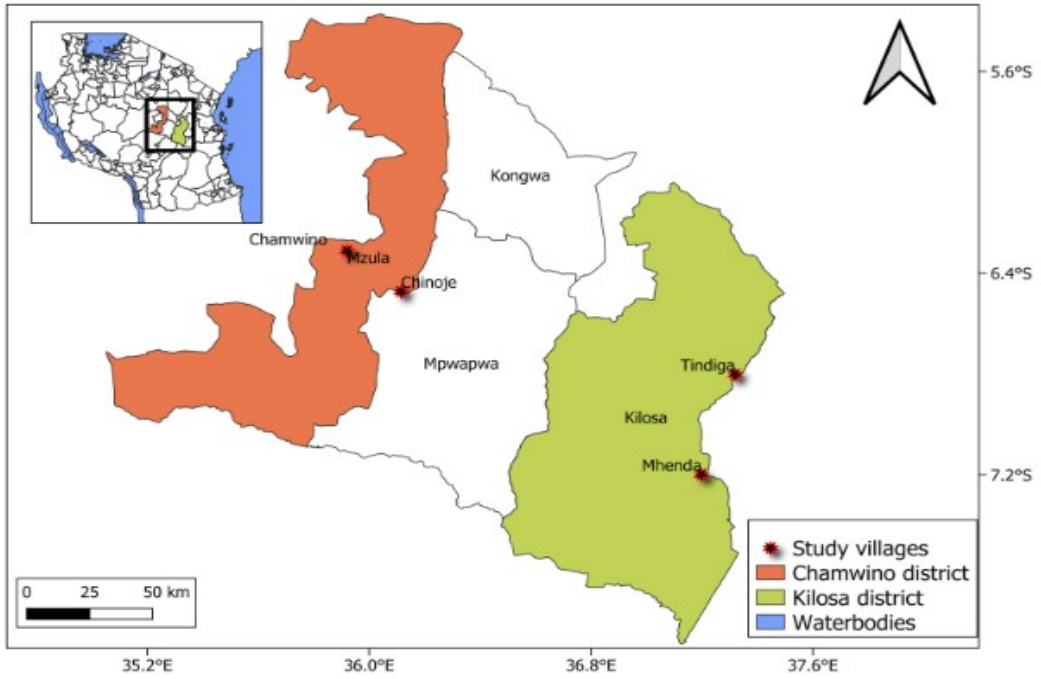

Figure 1.3: A map of study areas

The study population were apparently healthy school age children (5-12 years) enrolled along with their mothers or caregivers. The school-age children were selected because they are particularly vulnerable to undernutrition as the priority of nutritional interventions in most developing countries focus on fetal development and children below five years.

Mothers or caregivers were involved because they are responsible for food preparation and serving in the household. Additionally, mothers of reproductive age contribute to micronutrient status to their foetus during pregnancy. Using Fisher's formula (Fisher *et al*., 1991) the total sample size of 669 households was computed (Appendix 2). Based on similarities in population size between the four study villages, an average sample size of 167 households per village was randomly assigned from the respective village registers. Randomization of households was done using the ENA for SMART software (version 2011). The inclusion criterion for households to participate in the study was having a mother or a caregiver and a schoolchild aged between 5–10 years. Households that missed the target school child were excluded from the study. The village and hamlet leaders supported in the admission of the mother's or caregiver's-children pairs to the study at the respective villages.

A total of 666 out of 669 study participants completed the questionnaires for baseline assessment. Households that participated in the baseline survey were 167 from Mzula and 166 from Chinoje villages in Dodoma; and 169 from Mhenda-Kitunduweta and 164 from Tindiga villages in Morogoro. After the implementation of an integrated home gardening intervention, a total of 579 out of 666 (86.9%) mothers or caregivers and 563 out of 666 (84.5%) school children completed the follow-up study in July to August 2018 as presented in the study flow diagram in Figure 4.1.

1.10.3 Data collection tools and methods

1.10.3.1 Socio-demographic and morbidity characteristics

Socio-demographic data such as age, sex and educational level of the children's mother or caregiver; data on morbidity occurrence and dietary intake was collected using pretested questionnaire (Appendix 1). Moreover, anthropometric measurements and laboratory data

were collected. The data collection staff included phlebotomist and field assistants who received training of the survey tools and participated in pre-testing of the tools before embarking on the survey. Errors in data collection were checked and corrected before leaving the field.

1.10.3.2 Anthropometric assessment

Anthropometric status was assessed using measurements of weight, height and mid-upper arm circumference (MUAC) according to the Nutrition Assessment, Counselling and Support (NACS) guide for training health facility-based service providers (FANTA, 2015). Briefly, body weight was measured in light clothing to the nearest 0.1 kg using a SECA electronic scale with a tare facility (SECA GmbH & co.kg, Hamburg, Germany). Height was measured to the nearest 0.1 cm using a stadiometer (Model S0114540, UNICEF, New York, NY, USA). The height measurement was taken while the child was standing without shoes on a horizontal flat plate attached to the base of the stadiometer with heels together, and stretched upwards to the full extent with the head looking straight ahead. The mid upper arm circumference (MUAC) between the acromion and the olecranon process (shoulder and elbow) was measured to the nearest 0.1 cm using standard MUAC measuring tapes for reference age (UNICEF). Anthropometric indices, height-for-age (HAZ), weight-for-age (WAZ), and body mass index-for-age (BMIA) Z-scores were computed using WHO AnthroPlus (v1.0.4) software; nutritional status indices for overweight (>+1SD BMI-for-age Z score), obesity (>+2SD BMI-for-age Z score), thinness/wasting (<-2SD of BMI-for-age Z score), underweight (<-2SD of weight-for-age Z score), and stunting (<-2SD of height for- age Z score) were defined according to the WHO reference growth charts for children aged 5 to 19 years (WHO, 2007).

1.10.3.3 Biochemical assessment

Phlebotomy technicians collected venous blood samples (3-5 ml) from each child using sterile safety multifly needles (butterfly) and serum monovettes, at the study site. Haemoglobin (Hb) concentrations were measured on site using Hemocue micro-cuvettes filled with a drop of whole venous blood and a portable battery-operated haemoglobinometer (HemoCueHb 201+, Angelhom, Sweden) for immediate reading. Anaemia was defined as Hb <115 g/L for children aged 5-11 years and < 120 g/L for children 12–14 years with adjustment for altitude as per WHO recommendation (WHO, 2017).

At the study sites, venous blood samples were centrifuged at 1850 × *g* for 15 minutes at room temperature, and serum aliquots were distributed and transferred to 2.0 ml Eppendorf tubes, frozen at -20°C at study sites, further transported to the laboratory at Sokoine University of Agriculture (SUA) in Morogoro for storage at -80°C, before finally being transported on dry ice to University of Hohenheim, Stuttgart for the analysis on serum micronutrients. Retinol (vitamin A), carotenoids (α-Carotene, β-Carotene, β-Cryptoxanthin, Lutein-zeaxanthin, Lycopene) and tocopherols (vitamin E) were determined using the high-performance liquid chromatography (HPLC). Serum aliquots were analysed at the VitMin Lab by Dr. JG Erhardt on iron status markers (ferritin, sTfR), infection or inflammation markers (CRP, AGP) by a sandwich enzyme-linked immune-sorbent assay (ELISA) technique and serum zinc by a spectrophotometric method as previously described in detail (Erhardt *et al*., 2004; Stuetz *et al*., 2019). Iron deficiency (ID) was defined as serum ferritin <15µg/L or sTfR >8.5 mg/L for children > 5 years (WHO, 2001) and total body iron stores (IST) were calculated by an equation using ferritin and sTfR (Cook *et al*., 2003); serum ferritin was adjusted for respective correction factors for children and the 3 different inflammation stages: factor 0.64 for incubation (CRP > 5

mg/L and AGP ≤ 1 g/L), 0.39 for early convalescence (CRP > 5 mg/L and AGP > 1 g/L), and 0.65 (CRP ≤ 5 mg/L and AGP > 1 g/L) for late convalescence (Thurnham *et al*., 2010) . Elevated acute phase proteins CRP > 5 mg/L and AGP >1 g/L were used as indicators for an acute phase response by infection or inflammation (Thurnham *et al*., 2010). Retinol <0.7 µmol/L was considered indicative of vitamin A deficiency (WHO, 1996) while serum zinc <0.65 mg/L was used to indicate low/deficient zinc status for all age and sex groups combined (Hotz *et al*., 2003). Vitamin E deficiency was considered when α-tocopherol was less than 11.6 µmol/L (MC Lobo *et al*., 2019).

1.10.3.4 Dietary intake

A 24-hour dietary recall method was applied to assess dietary intake of the target child. Mothers or caregivers responsible for preparing food assisted their children to recall all food items including ingredients for mixed dishes, snacks and beverages consumed over a period of 24 hours preceding day of the survey. Household utensils and measuring aids including a set of plates, bowls, spoons and measuring cups were used to help mothers/caregivers estimate portion sizes. The 24-hour macro- and micronutrient intake (vitamins and minerals) of all reported foods were calculated per amount (grams, litres) for each child using the 'NutriSurvey' software package (Erhardt, 2007). This software contains all reported foods and recipes listed in the Tanzanian food composition tables (Lukmanji *et al*., 2008) and additionally micronutrient contents of indigenous leafy vegetables collected in the study area (Gowele *et al*., 2019) were entered and processed. The adequacy of micronutrient intake was determined using the recommended nutrient intakes (RNIs) by WHO and FAO (WHO and FAO 2004). For macronutrients, the percentage of total energy intake from proteins, carbohydrates and total fat was evaluated using the following acceptable macronutrient distribution ranges (AMDR) for children 4 to

18 years of age: 10–30% for protein, 25–35% for fat and 45–65% for carbohydrate (IoM, 2005).

1.10.3.5 Leafy vegetable sample collection, preparation and laboratory analysis

Thirteen different species of indigenous leafy vegetables (ILVs) were collected from their natural habitat during wet season in April 2016 (Kilosa samples) and March 2017 (Chamwino samples). Selection of the vegetable species for this study was based on the focus group discussion findings in the study areas where most women mentioned their wider consumption by the community. Identification of the vegetable samples was done by a botanist from the Department of Crop Science and Horticulture at Sokoine University of Agriculture (SUA).

The leafy edible parts of the vegetables were separated from the main plant, placed in black polyethylene bags and subsequently transported to the laboratory at SUA. The first batch of samples from Kilosa were kept from April 2016 to August 2016 in a -30°C freezer then transported on dry ice to the University of Hohenheim, Stuttgart in Germany; stored at -80°C and analysed in September 2016. The second batch of samples from Chamwino were kept for two days in a -30°C freezer then immediately transported on dry ice to the University of Hohenheim, Stuttgart in Germany; stored at -80°C for one week till analysis were done. The individual ILV species from both sample batches were freeze-dried for 24 hours (protected from light using aluminium foil) using Azbil-Telstar freeze drier (2014 model LyoQuest; Azbil-Telstar, Spain), ground in a mortar to a fine homogenous powder and stored in airtight containers protected from light until individual analysis on micronutrients and phytate content were done.

All determinations were done in freeze-dried samples (powder). Carotenoids (Lutein/Zeaxanthin, ß-Cryptoxanthin, α-Carotene and ß-Carotene), tocopherols, and ascorbic acid (AA) were analysed using High-Performance Liquid Chromatography (HPLC). The minerals iron, calcium, magnesium, zinc, and phosphorus were determined using Inductively Coupled Plasma-Optical Emission Spectrophotometry (ICP-OES), while phytic acid content was analysed after clean-up using a photometric method. Micronutrient and phytate contents in homogenized freeze-dried ILV samples were determined as milligrams per 100 g dry matter (mg/100 g). Details regarding botanical names, local names, collection points and the conversion factors of the analysed ILVs are given in chapter 3, paper two of this thesis.

1.10.3.6 Home gardening awareness and practice

This section of home gardening comprised questions to test the availability of different kinds of fruits and vegetables in the study areas, their consumption and the corresponding home gardening practices. The collected information related to home gardening included: (i) The respondent grow vegetables in home gardens (yes or no), (ii) Respondent awareness of sack or pocket gardens as a method of growing vegetables (yes or no) which was measured by mothers or caregivers ability to correctly describe how it is practiced and, (iii) Respondent grow vegetables in a sack or pocket garden (yes or no). Improvements in the above indicators were expected to translate into increased vegetable consumption that was measured using a 24-h recall method. Measures included a pre- post assessment to analyze the vegetable intake among participants before and after the home gardening intervention is implemented. Data was collected through face to face interviews using questionnaires administered by field assistants. A total of 666 sampled mothers or caregivers participated in the baseline survey (pre-intervention) and 579 during the follow up (post-intervention) as presented in chapter four of this thesis.

1.10.3.7 Design and implementation of home gardening intervention

A community-based participatory approach was applied in implementing the integrated home gardening intervention in the study areas. The initial step involved dissemination of baseline research results to men and women from participating households. Thereafter, mapping of factors that affect the vegetable intake and eventually micronutrient status of the study population was done in a participatory manner. This step was followed by involvement of both men and women in the plan of home gardening intervention as a measure to improve vegetable intake and eventually micronutrient status of the study population.

The home garden intervention involved five key activities: 1) introduction of the home garden intervention to the participating households and village officials, 2) participatory selection of demonstration sites, 3) preparation of training materials and farm inputs (fliers, polyethylene sacks, seeds), 4) training on compost manure preparation and starting of vegetable nurseries and 5) training on how to set up a garden. Men and women from the participating households were trained on how to set up three kinds of home gardening systems; these are, sack or pocket, raised bed and brick gardens. However, due to the water scarcity in the study areas, the sack or pocket gardens are the focus of this study. The use of pocket or sack garden employ minimal use of water thus has the potential to overcome water scarcity. Other potentials of pocket gardens include the use of easily accessible materials such as sand, pebbles, animal manure, and soil that can be found within the localities. Training and demonstrations on the use of pocket gardens in growing vegetables was done using a participatory approach where household members were grouped by hamlets with maximum 30 households per session. The garden training sessions concentrate on material preparation, land preparation and garden layout, raised planting bed preparation, nursery bed preparation and sowing practices, watering of vegetables,

proper fencing using locally available materials, fertilizer application, weeding, insects and disease management and harvesting of vegetables.

During the training sessions each participating household received polyethylene bags and vegetable seeds (spinach, amaranths and Chinese cabbage) selected for high nutrient content, ease of growing and participant's acceptance. Vegetable seeds were planted in the holes on the sides of the pockets and on the top. In particular, the amaranths seeds were planted at the bottom surrounding the earth-filled pocket to utilize the water that trickles down. The pockets were placed within the homestead of the participating households. Each earth-filled pocket contained up to 48 plants of spinach or Chinese cabbage that could be harvested up to 3 months. It took 28 days for spinach and 21 days for Chinese cabbage and amaranths to be ready for harvest. During the implementation phase, participants received a weekly follow-up visit from local field officers. Four field officers one in each study village was hired by the project from October 2017 to May 2018 to monitor the implementation and progress of the intervention. Detailed description of the home gardening intervention is presented in chapter four of this thesis.

1.10.3.8 Design and implementation of school gardens

The school gardens were implemented in all five public primary schools situated in the study villages. The sited areas for school gardens were used as demonstration plots for training on three gardening systems (pocket, raised bed and brick gardens). Training was conducted to school teachers and pupils. The training sessions for school teachers and pupils were done separately from the ones done to household members and village leaders. Fifteen Pupils from each class (grades 4-7) were selected by their teachers to participate in the training sessions as representatives of their fellow pupils to become peer trainers. The training included topics similar to the ones presented in home gardening intervention.

Likewise, polyethylene pockets or bags and vegetable seeds for Swiss chard, Chinese cabbage and amaranths were provided to schools upon completion of the training session. Hybrid mango and papaya seedlings from horticulture unit of Sokoine University of Agriculture were additionally distributed and planted in respective schools during the following rainy season. However, due to water scarcity the implementation phase of school gardens was delayed to allow the installation of rain water harvesting systems and storage tanks in each school. For this reason, the possible impacts of school garden intervention would have been realized beyond the study period and thus not assessed during the follow up survey.

1.10.3.9 Data analysis

Data were entered and analysed using SPSS software (SPSS Inc., Chicago, IL, USA; Version 20.0.0) and p values <0.05 were considered as statistically significant. Continuous variables that were normally distributed were described using mean and standard deviation (SD) whereas the non-normally distributed variables by their median and interquartile range (IQR). Categorical and binary data were presented using percentages and frequencies (number). Socio-demographic characteristics, anthropometrics (weight), blood biomarkers (haemoglobin, acute phase proteins, and serum micronutrients), and data on the dietary intake of micronutrients of the study participants were described using medians with interquartile range (IQR), mean (SD), and frequencies (number), as appropriate. For comparisons between the study villages and districts, the Kruskal–Wallis, Mann–Whitney U-test, and ANOVA (post hoc Scheffe test) for continuous variables and Chi-squared tests for prevalence and categorical data were used. Multiple logistic regression analysis with a forward (stepwise) approach was applied to identify independent risk factors of stunting (HAZ < −2SD), underweight (WAZ < −2SD), overweight (BMI > 1SD), anaemia

(haemoglobin < 115 g/L), iron deficiency (ferritin < 15 µg/L or sTfR > 8.5 mg/L), and vitamin A deficiency (serum retinol < 0.7 µmol/L) in the two districts.

The concentrations of carotenoids, vitamins, minerals and phytic acid in ILVs were given per wet weight (fresh weight) and described as means and standard deviations. Data used for provitamin A carotenoids, ascorbic acid and tocopherols were means of triplicate (n = 3) determinations while for minerals and phytate were of duplicate (n = 2) determinations. Multiple comparisons of means between species of ILVs were performed by one-way analysis of variance (ANOVA) and post-hoc Tukey's HSD (Honestly Significant Difference) test; statistical significance was considered at a p value < 0.05.

Comparisons between the baseline and post intervention phase were done using the chi-squared and McNemar test for categorical data and the prevalence of deficiencies whereas for continuous variables the Wilcoxon Signed Ranks test was used. Logistic regression analysis was carried out to investigate the factors that predict anaemia and micronutrient deficiencies. In separate models anaemia, iron deficiency and zinc deficiency were included as dependent variables while acute phase proteins (AGP >1 g/L, CRP >5 mg/L), serum micronutrients, dietary micronutrient intake and home garden practice indicators were included as independent variables. A forward stepwise approach was used and only variables with P values < 0.05 were retained in the final models. The strength of association between potential predictive factors and nutrition status were measured by the Odds Ratio (OR) and their 95% confidence interval (CI). Appropriate fit of logistic regression models was confirmed using the Hosmer–Lemeshow goodness-of-fit test.

1.10.3.10 Ethical considerations

This study was approved by National Institute of Medical Research of Tanzania with reference number (NIMR/HQ/R.8a/Vol.IX/2226), and the Ethics Committee Landesärztekammer Baden-Württemberg, Stuttgart, Germany (F-2016-049). Permission was also obtained from the district administration and village leaders of the respective communities. The purpose of the study was explained to the eligible household heads including mother or caregiver of each child and signed or thumb-printed informed consent (Appendix 4) was obtained from each mother or caregiver. Notably, dissemination of research results to the study participants was done immediately after the baseline study.

1.11 Organization of the Thesis

This thesis contains five chapters. The first chapter introduces the basic concepts related to the study including the nutrition situation of school children, micronutrient functions and consequences of their deficiency, dietary patterns of school children, and contribution of indigenous leafy vegetables to micronutrient intake and importance of home gardens for improving nutrition situation. The first chapter further describes the meaning of key concepts used, the conceptual framework underlying the study and the general methodology used in the collection and analysis of data. Chapter two presents the first paper which addresses the first and second objectives of the study. In this paper, results of nutrition status, the associated factors and dietary intake of school children are presented. Chapter three presents the second paper which addresses the third objective of the study. The second paper present results of the micronutrient composition of selected indigenous leafy vegetables commonly consumed in the study areas. Chapter four presents the third paper in form of a publishable manuscript which addresses the fourth objective of the study. The publishable manuscript present results of the pre and post analysis of home gardening intervention on anthropometrics, haemoglobin, micronutrient intake and status

of school children in the study areas. Chapter five presents the general conclusions that summarize the major findings of this study, recommendations, limitations of the study, contribution of the study to the body of knowledge and areas for further research.

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CHAPTER TWO

PAPER ONE

nutrients

MDPI

Article

High Prevalence of Stunting and Anaemia Is Associated with Multiple Micronutrient Deficiencies in School Children of Small-Scale Farmers from Chamwino and Kilosa Districts, Tanzania

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Abstract: Inadequate macro- and micronutrient nutrition and its consequences, such as anaemia, iron and vitamin deficiency, and growth retardation, could particularly affect children of small-scale farmers. In the present cross-sectional study, 666 school children aged 5–10 years from villages of Chamwino and Kilosa districts were studied for associations between nutritional and micronutrient status and dietary intake. The overall prevalence of stunting, underweight, and overweight was 28.1, 14.4, and 5%, while that of anaemia and deficiency of iron (ID), vitamin A (VAD), and zinc (ZnD) was 42.9, 29.3, 24.9, and 26.4%, respectively. Dietary recalls (24h) revealed that, except of iron (74%), only small proportions of children reached the recommended daily micronutrient intakes: 4% for zinc, 19% for vitamin A, and 14–46% for B vitamins. Stunting was highly associated with wasting in both districts and with VAD in Chamwino. Anaemia was predicted by ID, VAD, and ZnD in Chamwino and by elevated infection markers, C-reactive protein (CRP) and α-1 glycoprotein (AGP), in Kilosa. Overall, elevated CRP and/or AGP increased the risk while higher serum carotenoids indicating a diet of more fruit and vegetables reduced the risk of VAD. The significantly lower prevalence of anaemia and ID in Chamwino was related to higher iron and vitamin A intake and the consumption of mainly bulrush millet with dark green leafy vegetables compared to maize or rice with legumes in Kilosa. Nutrition and hygiene education integrated with home and school garden programmes could reduce the multiple burdens of anaemia; micronutrient deficiencies and infections; and, in the long term, the prevalence of stunting.

Keywords: school children; stunting; anaemia; vitamin A; iron; micronutrient deficiency; Tanzania

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1. Introduction

Micronutrient malnutrition, especially of vitamin A, iron, and zinc, are common among children and remains one of the major public health challenges in developing countries [1]. Malnutrition in children can result both from a diet that is poor and deficient in essential macro- and micronutrients, and from the inefficient utilization of available nutrients due to infections and parasitic infestations [2,3].

In rural Tanzania, stunting, anaemia, iron, and vitamin A deficiency are among the most prevalent nutritional problems in school children [4–8]. Studies reported a high prevalence of stunting (21–79%), anaemia (29–80%), and deficiency of iron (33%) and vitamin A (32%), depending on the study area (Northwestern, Central, or Southern Tanzania), the age group of the children, and the year the study was conducted (from 1996 to 2015). However, all these studies as well the Tanzanian national demographic,

health, and nutrition surveys [9,10], missed a detailed nutritional assessment regarding micronutrient intake and status; further, the national surveys only focused on young children below 5 years of age, but malnutrition persists into the preadolescent period affecting school performance and reproductive health during puberty, particularly when child-bearing occurs early in life [11].

Stunting is a form of chronic malnutrition that reflects failure to receive adequate nutrition over a long period due to poor diets, recurrent infections, and chronic illness [12]. The long-term effects of stunting in individuals includes diminished cognitive and physical development; reduced productive capacity; and increased risk of degenerative diseases, such as diabetes. In addition, stunted children experience rapid weight gain and thus have an increased risk of becoming overweight or obese later in life [13].

Anaemia remains a major public health challenge among school children in Tanzania where infectious diseases, such as malaria and soil-transmitted helminths (STH), are highly prevalent [4,6]. Micronutrient deficiencies, especially of iron, vitamin A, folate, and vitamin B12, usually occur simultaneously and in conjunction with micronutrient-poor diets, which then leads to anaemia and other deficiency symptoms via synergistic effects [14,15].

The present study is part of the Scale-N project [16] aiming to achieve food and nutrition security of small-scale farmers in Dodoma and Morogoro regions by the support and development of nutrition-sensitive agricultural production, the establishment of pocket gardens for the production and consumption of leafy vegetables, and the improvement of nutritional behaviour [17]. At the baseline study in 2016, school children (in pre- and primary school aged 5–10 years) of four different rural villages in the Chamwino and Kilosa districts were enrolled along with their mothers to study (1) their nutritional and micronutrient status; (2) dietary intake in regard to the adequacy of micronutrients; and (3) determinants of stunting, wasting, anaemia, and iron- and vitamin A deficiency.

2. Materials and Methods

2.1. Study Area and Population

This cross-sectional survey was part of the baseline study under the Scale-N project conducted during the period July–August 2016 in Dodoma and Morogoro regions, Tanzania. Two districts in the regions of Dodoma and Morogoro were purposively selected due to their high prevalence of anaemia in children aged 6–59 months, as shown in the national 'Tanzania Demographic and Health Survey' [10]. The semi-arid Chamwino district (350–500 mm annual rainfall) is one of seven districts of the Dodoma region and consists of primarily flat plains; the predominantly sub-humid Kilosa district (600–800 mm annual rainfall) is one of seven districts of the Morogoro region and is characterized by flat plains, highlands, and dry alluvial valleys. Briefly, the food system in Chamwino is primarily based on millet and sorghum with a deep attachment to livestock [18,19]. Other crops commonly cultivated include sunflower, groundnuts, and bambara nuts. In the Kilosa district, legumes, rice, sorghum, maize, and horticultural crops are common, with some livestock integrated into the livelihood system [18].

The households were sampled from four villages participating in the Scale-N project, Mzula and Chinoje in the Chamwino district, and Tindiga and Mhenda-Kitunduweta in the Kilosa district. The villagers and study population were practically all self-sufficient small-scale farmers. The original plan was to include at least 150 households with associated school-age children (6–9 years) in each of the 4 villages. More than 165 households per village with the inclusion criterion of having a mother and a corresponding school-age child were randomly assigned from the village register (using ENA for SMART software version 2011). A total of eligible 669 households with mothers or caregivers and apparently healthy school children aged 5 to 10 years were successfully enrolled in the study. The survey was carried out according to the guidelines laid out in the Declaration of Helsinki and approved by the National Institute for Medical Research and the Ministry of Health, Community Development, Gender, Elderly and Children in Dar es Salaam, Tanzania (NIMR/HQ/R.8a/Vol. IX/2226) and the Ethics Committee Landesärztekammer

Baden-Württemberg, Stuttgart, Germany (F-2016-049). Written informed consent was obtained from 669 mothers and/or caregivers for the analysis of nutritional status (anthropometry and dietary intake) and the collection of blood for the determination of serum micronutrients; three households with mother–child pairs were excluded from the analysis due to missing blood draw or uncompleted questionnaires, giving a total sample size of 666 school children within the baseline survey.

2.2. Socio-Demographic Information

Socio-demographic information (personal data) and dietary intake patterns of the study population were collected using the pre-tested structured Scale-N project survey tool. The questionnaire was interview based and prepared in both English and Swahili version, while the Swahili version was the one posed during the interview. All enumerators including those who took the anthropometric measurements received intensive training prior the survey following the guidelines endorsed by FANTA III, Training Package for Facility-Based Service Providers. The questionnaire also included questions about mother/caregiver literacy; household size; and information on the child's age, sex, and reported malaria (in the last 3 months) and diarrhoea (in the last 4 weeks).

2.3. Anthropometric Assessment

Body weight was measured in light clothing to the nearest 0.1 kg using a SECA electronic scale with a tare facility (SECA GmbH & co.kg, Hamburg, Germany). Height was measured to the nearest 0.1 cm using a stadiometer (Model S0114540, UNICEF, New York, NY, USA). The height measurement was taken while the child was standing without shoes on a horizontal flat plate attached to the base of the stadiometer with heels together, and stretched upwards to the full extent with the head looking straight ahead. The mid upper arm circumference (MUAC) between the acromion and the olecranon process (shoulder and elbow) was measured to the nearest 0.1 cm using standard MUAC measuring tapes for reference age (UNICEF). Anthropometric indices, height-for-age (HAZ), weight-for-age (WAZ), and body mass index-for-age (BMIA) Z-scores were computed using WHO AnthroPlus (v1.0.4) software; nutritional status indices for overweight (>+1SD BMI-for-age Z score), obesity (>+2SD BMI-for-age Z score), thinness/wasting (<−2SD of BMI-for-age Z score), underweight (<−2SD of weight-for-age Z score), and stunting (<−2SD of height-for-age (HAZ) Z score) were defined according to the WHO reference growth charts for children aged 5 to 19 years [20].

2.4. Dietary Assessment

A single 24 h dietary recall method was applied to assess the dietary intake of the target child. We calculated the 24 h macro- and micronutrient intake (vitamins and minerals) of all reported foods per amount (grams and litres) for each child using the 'NutriSurvey' software package [21]. This software contains all reported foods and recipes listed in the Tanzanian food composition tables [22], and, additionally, the micronutrient contents of indigenous leafy vegetables collected in the study area [23] were entered and processed. We analysed the adequacy of micronutrient intake using the recommended nutrient intakes (RNIs) by WHO [24]. For macronutrients, the percentage of total energy intake from proteins, carbohydrates, and total fat was evaluated using the following acceptable macronutrient distribution ranges (AMDR) for children aged 4 to 18 years: 10–30% for protein, 25–35% for fat, and 45–65% for carbohydrate [25].

2.5. Blood Sampling and Analysis

Venous blood (3–5 mL) was drawn from each target child using sterile safety multifly needles (butterfly) and serum monovettes. Haemoglobin (Hb) concentrations were measured immediately on site by transferring a drop of venous blood taken from the sterile safety multifly into microcuvettes and measuring it with a portable battery-operated

haemoglobinometer (HemoCueHb 201+, Angelhom, Sweden). Anaemia was defined as Hb < 115 g/L for children aged 5–11 years [26].

Venous blood samples were centrifuged at 1850× *g* for 15 min at room temperature, and serum aliquots were distributed and transferred to 2.0 mL Eppendorf tubes, frozen at −20 °C at study sites, and further transported to the laboratory at Sokoine University of Agriculture (SUA) in Morogoro for storage at −80 °C, before finally being transported on dry ice to the University of Hohenheim, Stuttgart, for the analysis on serum micronutrients. Retinol (vitamin A), carotenoids, and tocopherols (vitamin E) were determined using high-performance liquid chromatography (HPLC), while serum aliquots were analysed (at the VitMin Lab, Dr. JG Erhardt, Willstaett, Germany) on iron status markers (ferritin; sTfR, soluble transferrin receptor), infection/inflammation markers (CRP, C-reactive protein; AGP, α-1 glycoprotein) by a sandwich enzyme-linked immune-sorbent assay technique, and serum zinc by a spectrophotometric method as previously described in detail [17,27]. Iron deficiency (ID) was defined as ferritin < 15 µg/L or sTfR > 8.5 mg/L for children >5 years [28], while total body iron stores (IST) were calculated by an equation using ferritin and sTfR [29]. Elevated acute phase proteins CRP > 5 mg/L and AGP > 1 g/L were used as indicators for an acute phase response by infection or inflammation [30], and serum ferritin was adjusted for respective correction factors for children and the 3 different inflammation stages: factor 0.64 for incubation (CRP > 5 mg/L and AGP ≤ 1 g/L), 0.39 for early convalescence (CRP > 5 mg/L and AGP > 1 g/L), and 0.65 for late convalescence (CRP ≤ 5 mg/L and AGP > 1 g/L). Retinol < 0.7 µmol/L was considered to be indicative of vitamin A deficiency (VAD) [31], while serum zinc < 0.65 mg/L was used to indicate low/deficient zinc status for children <10 years [32]; serum zinc was not adjusted according to the BRINDA analysis study.

2.6. Statistical Analysis

Socio-demographic characteristics, anthropometrics (weight), blood biomarkers (haemoglobin, acute phase proteins, and serum micronutrients), and data on the dietary intake of micronutrients of the study participants are described using medians with interquartile range (IQR), mean (SD), and frequencies (number), as appropriate. For comparisons between the study villages and districts, the Kruskal–Wallis, Mann–Whitney U-test, and ANOVA (post hoc Scheffe test) for continuous variables and Chi-squared tests for prevalence and categorical data were used. Multiple logistic regression analysis with a forward (stepwise) approach was applied to identify independent predictors of stunting (HAZ < −2SD), underweight (WAZ < −2SD), overweight (BMI > 1SD), anaemia (haemoglobin < 115 g/L), iron deficiency (ferritin < 15 µg/L or sTfR > 8.5 mg/L), and vitamin A deficiency (serum retinol < 0.7 µmol/L) in the two districts. The following co-variables were assessed in the initial and separate models: age, sex, reported malaria (in the last 3 months) or diarrhoea (in the last 4 weeks), family size (number of persons in the HH), mother's literacy status (can read and write vs. not), accompanying malnutrition (HAZ < −2SD, WAZ < −2SD, BMI > 1SD), anaemia (yes = 1 vs. no = 0), MN deficiencies (of iron, vitamin A, or zinc; yes = 1 vs. no = 0), serum micronutrients (e.g., β-carotene), elevated acute phase proteins CRP or AGP, and sufficient dietary intakes of assessed MNs (e.g., iron or vitamins ≥ RNI); probability for the stepwise entry and removal were set at $p < 0.05$ and $p < 0.10$, respectively; appropriate fit of the logistic regression models was confirmed using the Hosmer–Lemeshow goodness-of-fit test. All statistical analyses were carried out using SPSS software (SPSS Inc., Chicago, IL, USA; Version 20.0.0), and p values < 0.05 were considered as statistically significant.

3. Results

Socio-demographic characteristics, anthropometry, and morbidity data of children were compared by village and are summarized in Table 1. The study included a total of 666 children (54.7% females) with a median (IQR) age of 7.3 (6.4, 8.1) years; almost every second mother and/or caregiver (43%) could not read and write. The overall stunting

prevalence was 28%, ranging from 25% in Chinoje to 35% in Mhenda-Kitunduweta village; the prevalence of underweight was 14%, ranging from 11% in Tindiga to 17% in Mzula village; overweight was more frequent in the Kilosa villages (7–8%) than in the Chamwino villages (2.4%). In agreement, the median MUAC and BMI-for-age Z-scores were significantly higher in Kilosa district than in Chamwino district. The highest proportion of overweight (7.9%) and stunting (34.8%) was in the Mhenda-Kitunduweta village, where the children had simultaneously the highest prevalence of reported malaria and diarrhoea in the weeks and months prior to the survey.

Table 1. Socio-demographic characteristics and anthropometrics of the children by village.

Village	All	Mzula	Chinoje	Tindiga	Mhenda-Kitunduweta	*p*
District		Chamwino			Kilosa	
Children, N	**666**	**167**	**166**	**169**	**164**	
Age, years [1]	7.3 (6.4, 8.1)	7.4 (6.5, 8.1)	7.1 (6.4, 8)	7.2 (6.4, 8)	7.3 (6.4, 8.3)	0.488
5y (4.6–5.9y) [2]	12.0 (80)	9.0 (15)	12.7 (21)	14.2 (24)	12.2 (20)	
6y (6.0–6.9y)	30.0 (200)	27.5 (46)	31.3 (52)	30.2 (51)	31.1 (51)	
7y (7.0–7.9y)	27.8 (185)	30.5 (51)	28.9 (48)	29.0 (49)	22.6 (37)	0.196
8y (8.0–8.9y)	19.7 (131)	20.4 (34)	20.5 (34)	13.0 (22)	25.0 (41)	
9y (9.0–10.1y)	10.5 (70)	12.6 (21)	6.6 (11)	13.6 (23)	9.1 (15)	
Sex, female = 1	54.7 (364)	53.9 (90)	53.0 (88)	58.0 (98)	53.7 (88)	0.791
Family size	5 (4, 7)	5 (4, 7) [a]	6 (5, 7) [b]	5 (4, 6.5) [a]	5 (4, 7) [a]	0.037
Mother literate, =1	56.9 (379)	55.1 (92)	56.0 (93)	52.1 (88)	64.6 (106)	0.117
HAZ [3]	-1.42 ± 1.05	-1.39 ± 1.0	-1.32 ± 1.10	-1.37 ± 1.06	-1.60 ± 1.03	0.089
Stunting	28.1 (187)	25.7 (43)	25.3 (42)	26.6 (45)	34.8 (57)	0.181
WAZ	-1.11 ± 0.95	-1.25 ± 0.93	-1.09 ± 1.03	-1.0 ± 0.94	-1.09 ± 0.91	0.099
Underweight	14.4 (96)	16.8 (28)	13.9 (23)	11.2 (19)	15.9 (26)	0.485
BAZ	-0.28 ± 0.78	-0.52 ± 0.73 [c]	-0.31 ± 0.88 [b,c]	-0.18 ± 0.73 [a,b]	-0.08 ± 0.71 [a]	<0.001
Wasting/Thinness	0.8 (5)	0.0 (0) [a]	3.0 (5) [b]	0.0 (0) [a]	0.0 (0) [a]	0.002
Overweight	5.0 (33)	2.4 (4) [a]	2.4 (4) [a]	7.1 (12) [b]	7.9 (13) [b]	0.007
Obese	0.2 (1)	0 (0)	0.6 (1)	0 (0)	0 (0)	0.389
Malaria, =1	30.7 (205)	16.7 (28) [a]	21.1 (35) [a]	34.9 (59) [b]	50.6 (83) [c]	<0.001
Diarrhoea, =1	20.7 (138)	18.5 (31)	22.9 (38)	16.0 (27)	25.6 (42)	0.127

Data are median (25th and 75th percentile) [1], percentage (number) [2], mean (SD) [3], and all such values. *p* values: Kruskal–Wallis, Chi-square test (prevalence) or One-Way ANOVA as appropriate. Villages not sharing a superscript letter ([a,b,c]) are significantly different ($p < 0.05$) to each other (Mann–Whitney U and Tukey HSD tests for continuous variables and Chi-square test for prevalence). MUAC, mid-upper arm circumference; HAZ, height-for-age Z-score; stunting, HAZ< −2SD; WAZ, weight-for-age Z-score; underweight, WAZ < −2SD; BAZ, BMI-for-age Z-score; wasting/thinness, BAZ< −2SD; overweight, 1SD < BAZ ≤ 2SD; obese, BAZ > 2SD [20]; malaria, reported in the last 3 months and diarrhoea, reported in the last 4 weeks.

The results of haemoglobin (Hb) and iron status (ferritin, sTfR), serum micronutrients (retinol, zinc, carotenoids, and tocopherols), and infection markers (CRP, AGP) by village are presented in Table 2. The median (IQR) haemoglobin was 116 (109, 125) g/L, and 42.9% of all children were anaemic (Hb < 115g/L). Children from villages in Kilosa district had significantly lower haemoglobin than those in Chamwino, and anaemia prevalence was more than twice as high (59.2 and 68.3%) than in children from villages in the Chamwino district (18.6 and 25.9%). The overall prevalence of elevated acute phase proteins was 11.7% for CRP and 22.3% for AGP. Children from Mhenda-Kitunduweta (Kilosa) had the highest and also a significantly higher prevalence of increased CRP and AGP compared to the other villages; simultaneously, children from this village had the highest median serum ferritin concentration, even after adjustment for stages of inflammation, but the highest median soluble transferrin receptor (sTfR). indicating tissue iron deficiency; Mhenda-Kitunduweta showed, excluding Tindiga (another village in Kilosa), the highest prevalence of iron deficiency (ID) among the four study villages. The overall prevalence of ID was 29.3%, and in the villages of Kilosa was significantly higher than in those from Chamwino (39.1 and 40.2% vs. 20.4 and 17.6%). The median serum zinc concentration was 0.723 mg/L, with 26.4% of all children and 39% of children from Chinoje showing deficiency (ZnD) or low serum values of zinc (<0.65 mg/L).

Table 2. Haemoglobin, infection (CRP and AGP) and iron status markers (ferritin and sTfR), and serum micronutrients in children by village.

Village	All	Mzula	Chinoje	Tindiga	Mhenda-Kitunduweta	*p*
District		Chamwino		Kilosa		
Children, N	666	167	166	169	164	
Haemoglobin (g/L) [1]	116 (109, 125)	126 (117, 132) [a]	120 (114, 126) [b]	112 (107, 119) [c]	110.5 (102, 117) [d]	<0.001
Hb < 115 g/L, % (n) [2]	42.9 (286)	18.6 (31) [a]	25.9 (43) [a]	59.2 (100) [b]	68.3 (112) [b]	<0.001
CRP >5 mg/L	11.7 (78)	6.0 (10) [a]	9.1 (15) [a]	10.1 (17) [a]	22.0 (36) [b]	<0.001
AGP > 1 g/L	22.3 (148)	10.8 (18) [a]	12.7 (21) [a]	28.4 (48) [b]	37.2 (61) [b]	<0.001
Ferritin (µg/L)	39.9 (28.5, 59.5)	37.7 (28.8, 56.4) [a]	36.3 (26.9, 55.2) [a]	33.7 (24.3, 47.1) [b]	53.45 (35.1, 82.2) [c]	<0.001
Ferritin, adj. (µg/L)	34.2 (24.7, 52.6)	36.3 (26.4, 54.4) [a,c]	34.4 (24.9, 53.8) [a]	28.0 (20.8, 41.9) [b]	42.6 (29.5, 57.6) [c]	<0.001
sTfR (mg/L)	7.17 (6.14, 8.65)	6.76 (5.72, 7.95) [a]	6.64 (5.79, 7.75) [a]	7.75 (6.56, 9.50) [b]	7.93 (6.63, 10.29) [b]	<0.001
ID, adjusted	29.3 (195)	20.4 (34) [a]	17.6 (29) [a]	39.1 (66) [b]	40.2 (66) [b]	<0.001
IST, adj. (mg/kg BW)	4.09 (2.55, 5.69)	4.79 (3.01, 6.10) [a]	4.51 (3.14, 6.10) [a]	3.16 (1.60, 4.68) [b]	4.15 (2.81, 5.64) [a]	<0.001
Zinc (mg/L)	0.723 (0.64, 0.80)	0.752 (0.68,0.84) [a]	0.666 (0.60, 0.75) [b]	0.723 (0.65, 0.80) [a,c]	0.723 (0.64, 0.80) [c]	<0.001
Zinc < 0.65 mg/L	26.4 (176)	16.8 (28) [a]	39.2 (65) [b]	23.1 (39) [a,c]	26.8 (44) [c]	<0.001
Retinol, µmol/L	0.853 (0.70, 0.99)	0.848 (0.72, 0.99) [a]	0.814 (0.63, 0.97) [b]	0.870 (0.75, 1.00) [a]	0.878 (0.69, 0.99) [a,b]	0.011
Retinol, < 0.7 µmol/L	24.9 (166)	22.8 (38) [b]	34.3 (57) [c]	16.0 (27) [b]	26.8 (44) [a,c]	0.001
γ-Tocopherol, µmol/L	0.706 (0.42, 1.18)	1.108 (0.83, 1.51) [a]	1.214 (0.81, 1.84) [a]	0.429 (0.28, 0.63) [b]	0.456 (0.35, 0.64) [c]	<0.001
α-Tocopherol, µmol/L	15.36 (13.2, 8.0)	14.45 (12.6, 16.3) [a]	13.88 (12.0, 15.8) [b]	17.57 (15.2, 20.3) [c]	16.26 (14.2, 18.7) [d]	<0.001
α-Carotene, µmol/L	0.110 (0.04, 0.29)	0.043 (0.03, 0.07) [a]	0.034 (0.02, 0.05) [b]	0.286 (0.19, 0.47) [c]	0.285 (0.18, 0.44) [c]	<0.001
β-Carotene, µmol/L	0.483 (0.33, 0.68)	0.484 (0.34, 0.67) [a]	0.554 (0.41, 0.76) [b]	0.434 (0.30, 0.63) [a]	0.451 (0.32, 0.64) [a]	<0.001
β-Cryptoxanthin, µmol/L	0.162 (0.07, 0.34)	0.334 (0.17, 0.51) [a]	0.077 (0.05, 0.13) [b]	0.095 (0.05, 0.23) [b]	0.240 (0.13, 0.50) [a]	<0.001
Lutein/zeaxanth., µmol/L	0.917 (0.62, 1.24)	1.176 (0.94, 1.50) [a]	1.174 (0.94, 1.52) [a]	0.689 (0.50, 0.96) [b]	0.648 (0.51, 0.83) [b]	<0.001
Lycopene, µmol/L	0.334 (0.19, 0.57)	0.228 (0. 14, 0.35) [a]	0.227 (0.15, 0.35) [a]	0.650 (0.49, 0.87) [b]	0.378 (0.21, 0.58) [c]	<0.001

All values are median (25th and 75th percentile) [1] or percentage (number) [2], all such values; *p*-values: Kruskal–Wallis test for continuous variables and Chi-square test for prevalence. Villages not sharing a superscript letter ([a,b,c,d]) are significantly different ($p < 0.05$) to each other (Mann–Whitney U test, Chi-square test, as appropriate). CRP, C-reactive protein; AGP, α-1 glycoprotein; sTfR, soluble transferrin receptor; ID, iron deficiency, if serum ferritin (adjusted) < 15 µg/L or sTfR > 8.5 mg/L; IST, total body iron stores.

Median serum retinol of all children was 0.853 µmol/L, and each fourth child had vitamin A deficiency (24.9% < 0.7 µmol/L); as with zinc, Chinoje was the village with the highest burden, with one in three children suffering from VAD (34.3%). The serum concentration of γ-tocopherol was higher, while α-tocopherol was lower in Chamwino than in Kilosa. The differences in carotenoids between the districts and individual villages were as follows: regarding pro-vitamin A carotenoids, α-carotene was higher in Kilosa than in Chamwino, β-carotene was similar in the villages except for higher concentrations in Chinoje, while β-cryptoxanthin was significantly higher in Mzula and Mhenda-Kitunduweta than in the other villages. Lutein-zeaxanthin was overall about twice as high, while lycopene was lower in Chamwino than in Kilosa villages.

The assessed macro- and micronutrient intakes by 24 h recalls are presented in Table 3. The minimum recommended energy intake for the respective age and sex categories was overall only reached by 10% of the surveyed children; the protein consumption was at the lower limit, fat was below the lower limit, while carbohydrate intake exceeded the upper limit of the acceptable macronutrient distribution ranges of energy intake.

Table 3. Calculated macro- and micronutrient intake from 24 h recalls of children by village.

Village	All	Mzula	Chinoje	Tindiga	Mhenda-Kitunduweta	RNI/AMDR
District		Chamwino		Kilosa		
Children, N	666	167	166	169	164	
Energy, EN (Kcal) [1] EN ≥ RNI, % (n) [2]	898 (579, 1296) 10.2 (68)	715 (469, 1003) [a] 3.6 (6) [a]	550 (370, 759) [b] 3.0 (5) [a]	1181 (865, 1505) [c] 17.2 (29) [b]	1215 (979, 1559) [c] 17.1 (28) [b]	1250–1975
Protein (g) EN by Protein (%)	20 (12.8, 32.4) 11 (9, 14)	16.9 (11.1, 25.7) [a] 12 (9, 14) [a]	12.7 (8.7, 17.1) [b] 12 (11, 14) [b]	25.3 (18.0, 37.8) [c] 9 (7, 12) [c]	32.2 (21.9, 48.9) [d] 11 (9, 14) [d]	10–30%
Fat (g) EN by fat (%)	19.2 (9.3, 29.1) 19 (13, 24)	13.7 (7.9, 23.9) [a] 22 (14, 29) [a]	7.2 (4.0, 13.7) [b] 14 (10, 21) [b]	25.6 (17.4, 41.2) [c] 20 (16, 25) [c]	24.0 (16.2, 35.1) [c] 18 (13, 24) [c]	25–35%
Carbohydrates (g) EN by CHO (%)	144.1 (81.2, 218) 71 (64, 76)	106.2 (68.9, 139) [a] 67 (59, 76) [a]	66.3 (42.4, 107) [b] 73.50 (66, 77) [b]	203.2 (154.8, 255) [c] 71 (66, 75) [c]	215.2 (170.9, 270)[c] 71 (64, 76) [c]	45–65%
vitamin A (µg) RE ≥ RNI, % (n)	183 (65, 392) 19.1 (127)	253 (121, 441) [a] 21.6 (36) [a]	255 (158, 424) [a] 22.3 (37) [a]	141 (21, 456) [b] 21.9 (37) [a]	90 (26, 236) [c] 10.4 (17) [b]	450/500
Vitamin E (mg) α-TE ≥ RNI, % (n)	0.8 (0.6, 1.6) 4.8 (32)	0.9 (0.2, 2.6) [a] 7.2 (12)	0 (0, 0.5) [b] 2.4 (4)	0.8 (0.4, 1.3) [c] 3 (5)	1.1 (0.8, 2.3) [a] 6.7 (11)	5/7
Vitamin B1 (mg) B1 ≥ RNI, % (n)	0.5 (0.3, 0.7) 24.9 (166)	0.5 (0.3, 0.8) [a] 24.0 (40) [a]	0.4 (0.3, 0.6) [b] 13.9 (23) [b]	0.4 (0.3, 0.6) [b] 17.2 (29) [a,b]	0.7 (0.5, 1.0) [c] 45.1 (74) [c]	0.6/0.9
Vitamin B2 (mg) B2 ≥ RNI, % (n)	0.5 (0.4, 0.7) 24.8 (165)	0.6 (0.4, 0.7) [a] 26.9 (45) [a]	0.6 (0.4, 0.8) [a] 28.3 (47) [a]	0.4 (0.2, 0.6) [b] 13.0 (22) [b]	0.5 (0.3, 0.8) [c] 31.1 (51) [a]	0.6/0.9
Vitamin B6 (mg) B6 ≥ RNI, % (n)	0.8 (0.6, 1.1) 46.2 (308)	0.7 (0.5, 0.9) [a] 38.9 (65) [a]	0.7 (0.5, 0.9) [a] 36.7 (61) [a]	0.8 (0.6, 1.0) [b] 46.7 (79) [a]	1.0 (0.7, 1.2) [c] 62.8 (103) [b]	0.6/1.0
B12 (µg) B12 ≥ RNI, % (n)	0 (0, 0.3) 14.0 (93)	0 (0, 0) [a] 6.0 (10) [a]	0 (0, 0) [a] 1.2 (2) [b]	0 (0, 0.9) [b] 21.3 (36) [c]	0.1 (0, 2.2) [b] 27.4 (45) [c]	1.2/1.8
Folic acid (µg) FA ≥ RNI, % (n)	201 (128, 291) 33.8 (225)	206 (134, 281) [a] 31.1 (52) [a,b]	174 (116, 237) [a] 21.7 (36) [b]	178 (117, 285) [a] 32.0 (54) [a]	258 (160, 363) [b] 50.6 (83) [c]	200/300
Ascorbic acid (mg) AA ≥ RNI, % (n)	7.8 (1.5, 30.2) 23.1 (154)	3.3 (0.9, 9.3) [a] 14.4 (24) [a]	1.6 (0.7, 6.9) [a] 12 (20) [a]	15.9 (5.9, 39.1) [b] 31.4 (53) [b]	24.5 (9.5, 47.1) [c] 34.8 (57) [b]	30/35
Calcium (mg) Ca ≥ RNI, % (n)	309 (133, 671) 26 (173)	471 (242, 801) [a] 32.9 (55) [a]	512 (322, 763) [a] 35.5 (59) [a]	155 (77, 341) [b] 13.0 (22) [b]	175 (86, 490) [b] 22.6 (37) [c]	600/700
Iron (mg) Iron ≥ RNI, % (n)	11.5 (7.6, 18.6) 73.7 (491)	15.9 (10.9, 22.7) [a] 89.2 (149) [a]	18.4 (13.1, 24.7) [a] 95.8 (159) [b]	7.2 (5.3, 9.6) [b] 46.7 (79) [c]	8.8 (6.2, 13) [c] 63.4 (104) [d]	6/9
Zinc (mg) Zinc ≥ RNI, % (n)	4.9 (3.5, 6,5) 4.7 (31)	4.7 (3.3, 6.6) [a] 4.8 (8)	4.4 (3.3, 5.9) [a] 4.8 (8)	4.7 (3.5, 6.3) [a] 1.8 (3)	5.5 (4.2, 8.1) [b] 7.3 (12)	10.3/11.3
Magnesium (mg) Mg ≥ RNI, % (n)	163 (101, 242) 80.3 (535)	186 (106, 283) [a] 79 (132)	158 (96, 251) [a,b] 78.9 (131)	141 (91, 211) [b] 76.9 (130)	175 (121, 249) [a] 86.6 (142)	73/100

Figures are median (25th and 75th percentile) [1] and percentage (number) [2], and all such values. RE, retinol equivalent; α-TE, α-tocopherol equivalent (=vitamin E equivalent). Cut-offs for total energy requirement, adjusted for age, gender (boys and girls) and moderate levels of habitual physical activity were taken from the FAO/WHO/UNU [24]. For macronutrients, the percentage total energy intake (%EN) from proteins, total fat, and carbohydrates was evaluated using the acceptable macronutrient distribution ranges (AMDR) following the Institute of Medicine [25]. RNI/day, recommended daily nutrient intake regarding micronutrient adjusted for age (4–6, 7–9 years), and gender following the WHO recommendations [33]; for zinc, the low bioavailability was applied, and for iron, the 10% bioavailability was applied. Differences between the villages were assessed using Kruskal–Wallis and Mann–Whitney U tests for continuous variables and a Chi-square test for prevalence; all p values are < 0.001, except for % RE ≥ RNI = 0.013, % α-TE ≥ RNI = 0.082, % B2 ≥ RNI = 0.001, % Zinc ≥ RNI = 0.122, % Mg ≥ RNI = 0.128, and magnesium [mg] = 0.002; villages not sharing a superscript letter ([a,b,c,d]) are significantly different ($p < 0.05$) to each other.

Children from Chamwino had a significantly lower intake of energy, protein, fat, and carbohydrates but at the same time a significantly higher intake of vitamin A, calcium, and iron than those from Kilosa. However, only 19, 26, and 74% of the children reached the recommended nutrient intake (RNI) for vitamin A, calcium, and iron, respectively. The intake of vitamin E was in particular low, with only 5% reaching the RNI. In Kilosa, the intake of almost all B-vitamins tended to be higher and of ascorbic acid (AA) was significantly higher than in the Chamwino villages; Mhenda-Kitunduweta was the village with the highest intake of B-vitamins and AA; however, on average, only each fourth child

reached the RNI for B1, B2, or AA, while only 14, 46, and 34% reached the RNI for B12, B6, and folate, respectively.

Calculated mineral intake regarding zinc was similarly low and of magnesium similarly high between the districts and villages; but only 5% reached the RNI for zinc, while three of four children were able to achieve the RNI of magnesium. In summary, energy and fat intake was too low, and vitamin E, zinc, and vitamin B12 were the most limiting micronutrients in the diets of studied children.

The micronutrient status and intake and frequency and quantities of consumed food items in the two districts are summarized in Table 4. Children from Chamwino had a clearly and significantly lower prevalence of anaemia, ID, and of elevated infection markers CRP or AGP, but a higher prevalence of VAD and ZnD than those from the Kilosa villages; further, serum β-carotene, lutein-zeaxanthin, and γ-tocopherol were higher, while α-carotene, lycopene, and α-tocopherol were lower in Chamwino than in Kilosa children. The results of biological markers were largely consistent with the calculated MN intakes: the intake of iron and RE was twice as high, while that of zinc and vitamin E was lower in Chamwino than in Kilosa; however, the supply of zinc and vitamin E was in both districts clearly insufficient (<5% reached the RNI). In Chamwino, children consumed most frequently dishes that comprised bulrush millet and dark green leafy vegetables (DGLV). In Kilosa, the more varied diet consisted of maize or rice in combination with higher quantities of either legumes, roots, or other vegetables compared to Chamwino. Fruits, meat, and fish were more frequently consumed in Kilosa, but overall, in both districts, it was rare (2–25%) or even the exception.

Table 4. Micronutrient status and micronutrient and food intake of children compared by districts.

Micronutrient Status	Chamwino, n = 333	Kilosa, n = 333	*p*
Haemoglobin, g/L	123 (115, 130)	104 (111, 117)	<0.001
Anaemia, % (n)	22.2 (74)	63.7 (212)	<0.001
CRP ↑ or AGP ↑, % (n)	13.8 (46)	35.4 (118)	<0.001
Iron-ST, adj (mg/kg BW)	4.64 (3.04, 6.09)	3.67 (2.07, 5.29)	<0.001
ID adj., % (n)	19.0 (63)	39.6 (132)	<0.001
Retinol, µmol/L	0.830 (0.67, 0.97)	0.877 (0.72, 0.99)	0.028
VAD, % (n)	28.5 (95)	21.3 (71)	0.032
Zinc, mg/L	0.709 (0.64, 0.80)	0.723 (0.64, 0.80)	0.186
ZnD, % (n)	28.0 (93)	24.9 (83)	0.367
α-Carotene, µmol/L	0.038 (0.03, 0.06)	0.286 (0.18, 0.46)	<0.001
β-Carotene, µmol/L	0.526 (0.38, 0.73)	0.446 (0.31, 0.63)	<0.001
β-Cryptoxanthin, µmol/L	0.147 (0.07, 0.36)	0.173 (0.07, 0.32)	0.841
Lutein-zeaxanthin, µmol/L	1.176 (0.94, 1.51)	0.669 (0.50, 0.89)	<0.001
Lycopene, µmol/L	0.227 (0.15, 0.34)	0.531 (0.33, 0.75)	<0.001
γ-Tocopherol, µmol/L	1.145 (0.82, 1.66)	0.444 (0.32, 0.64)	<0.001
α-Tocopherol, µmol/L	14.15 (12.3, 16.1)	16.86 (14.7, 19.6)	<0.001
Micronutrient intake			
Iron intake, mg	17.2 (12.2, 24.3)	8.0 (5.8, 11.0)	<0.001
Iron suff. % (n)	92.2 (317)	55.0 (183)	<0.001
RE intake, µg	255 (151, 438)	104 (23, 308)	<0.001
RE suff. % (n)	21.9 (73)	16.2 (54)	0.061
Zinc intake, mg	4.49 (3.32, 6.19)	5.19 (3.76, 7.1)	0.006
Zinc suff. % (n)	4.8 (16)	4.5 (15)	0.854
α-TE intake, mg	0.32 (0.02, 1.60)	0.77 (0.45, 1.51)	<0.001
α-TE suff., % (n)	4.8 (16)	4.8 (16)	1.000

Table 4. *Cont.*

Food Intake	% (N)	Grams	% (N)	Grams	*p*
Millet [1]	92 (307)	375 (250, 500) *	4 (14)	250 (181, 375)	<0.001
Maize [2]	11 (36)	250 (190, 500)	86 (288)	250 (131, 400)	<0.001
Rice (with oil) [3]	3 (11)	205 (125, 300)	61 (203)	250 (250, 300)	<0.001
DGLV [4]	92 (305)	126 (90, 219)	31 (104)	100 (63, 150)	<0.001
Vegetables [5]	8 (26)	95 (49, 131)	27 (90)	100 (50, 125)	<0.001
Legumes [6]	53 (177)	20 (10, 93)	77 (258)	125 (100, 211) **	<0.001
Roots [7]	1 (5)	83 (32, 106)	26 (88)	168 (100, 200) *	<0.001
Fruits [8]	10 (35)	153 (115, 200)	18 (59)	182 (100, 300)	0.008
Meat [9]	4 (14)	75 (49, 115)	14 (47)	75 (50, 100)	<0.001
Fish [10]	2 (8)	95 (49, 107)	25 (85)	125 (72, 150)	<0.001

Data are median (25th and 75th percentile) or percentage (number); *p*-value: Mann–Whitney U test for continuous variables, Chi-square test for prevalence (e.g., % MN deficiency); * $p < 0.05$; ** $p < 0.001$ for food intake data in grams (Mann–Whitney U test); N = 332/333 serum samples in Chamwino. CRP ↑ or AGP ↑, CRP > 5 µg/L or AGP > 1 g/L. Iron-ST adj., total body iron stores adjusted (for ferritin); ID adj., iron deficiency if serum ferritin (adjusted) < 15 µg/L or sTfR > 8.5 mg/L. [1] Millet includes pearl and finger millet dishes; [2] maize includes stiff porridge 'Ugali' and soft porridge dishes; [3] rice cooked with coconut or oil and onions; [4] dark green leafy vegetables (DGLV) include 'Mlenda' or 'Ilende', amaranth, cow pea, sweet potato, and pumpkin leaves or spinach; [5] vegetables: okra, pumpkin, tomato, African eggplant, Chinese, or white cabbage; [6] legumes include beans, peas, bambara nut, and ground nut; [7] roots include cassava, potatoes, and yams; [8] fruits include banana, baobab, guava, mango, papaya, and water melon; [9] meat includes beef, goat, chicken, and pork; [10] fish includes fish relish and dried sardines.

Multiple logistic regression models to identify factors that are significantly associated with (1) stunting, underweight, and overweight, and with (2) anaemia, ID, and VAD are summarized in Tables 5 and 6. In both districts, stunting was highly associated with underweight (odds ratio of 24 and 29) and in Chamwino with VAD and younger age. In both districts, older and stunted children were at higher risk, while increasing MUAC was associated with a lower risk of underweight; in agreement with this, MUAC was positively associated while age was inversely associated with overweight. Notable, the positive association of overweight with ZnD in Chamwino, and the higher risk of overweight with ID, serum lutein-zeaxanthin, and higher vitamin B6 intake in Kilosa (Table 5).

Table 5. Determinants of stunting, underweight, and overweight in Chamwino and Kilosa district.

	Chamwino			Kilosa		
	HAZ < −2SD (25.5%) N = 85	WAZ < −2SD (15.3) N = 51	BMI > 1SD (2.7%) N = 9	HAZ < −2SD (30.6%) N = 102	WAZ < −2SD (13.5%) N = 45	BMI > 1SD (7.5%) N = 25
Age, years	0.71 (0.54, 0.94)	2.94 (1.77, 4.88) *	0.36 (0.14, 0.97)		1.93 (1.26, 2.98)	0.32 (0.17, 0.60) *
WAZ < −2SD	23.8 (10.7, 53.0) *			29.2 (11.0, 77.0) *		
HAZ < −2SD		30.5 (10.7, 86.5) *			20.4 (6.97, 58.9) *	
MUAC, cm		0.11 (0.05, 0.23) *	7.87 (3.10, 20.0) *		0.18 (0.10, 0.33) *	7.20 (3.74, 13.8) *
ID adj., =1 (yes)						4.53 (1.37, 15.0)
VAD, =1 (yes)	2.09 (1.12, 3.90)					
ZnD, =1 (yes)			6.46 (0.99, 42.18)			
β-Cryptoxanthin, µmol/L		0.10 (0.01, 0.75)				
Lutein-zeaxanth., µmol/L						5.91 (1.32, 26.5)
B6 intake ≥ RNI, =1						12.3 (2.24, 68.0)
R^2 (Nagelkerke)	0.369	0.640	0.546	0.298	0.613	0.576

Multiple logistic regression models with a forward approach; values are Exp (B) = odds ratio, and 95% CI; all factors at $p < 0.05$, except * $p < 0.001$. ID adj. = 1: iron deficiency (ferritin adjusted), yes vs. no = 0; VAD = 1: vitamin A deficiency, yes vs. no = 0; ZnD = 1: zinc deficiency, vs. no = 0. Variables in the initial models included age, sex, reported malaria (in the last 3 months) or diarrhoea (in the last 4 weeks), family size (number of persons in the HH), mother's literature status (can read and write = 1), HAZ < −2SD, WAZ < −2SD, anaemia (=1), MN deficiencies: ID, VAD, ZnD, serum micronutrients (e.g., β-carotene), and sufficient dietary intakes of assessed MNs (e.g., iron or vitamins ≥ RNI). HAZ < −2SD, stunting; WAZ < −2SD, underweight; BMI > 1SD, overweight; VAD = 1, vitamin A deficiency, ID = 1, iron deficiency, ZnD = 1, zinc deficiency.

Table 6. Determinants of anaemia, iron deficiency (ID), and vitamin A deficiency (VAD) in Chamwino and Kilosa districts.

	Chamwino			Kilosa		
	Anaemia (22.3%) N = 74	ID (19.0%) N = 63	VAD (28.5%) N = 95	Anaemia (63.7%) N = 212	ID (39.6%) N = 132	VAD (21.3%) N = 71
Malaria, =1		2.43 (1.28, 4.62)				
High CRP or AGP, =1			4.02 (1.95, 8.27) *	2.02 (1.22, 3.34)	1.84 (1.17, 2.91)	2.54 (1.43, 4.52)
ID, =1 (yes)	1.90 (1.00, 3.64)		2.39 (1.29, 4.44)			
VAD, =1 (yes)	2.05 (1.16, 3.62)	2.79 (1.57, 4.97) *				
ZnD, =1 (yes)	2.19 (1.26, 3.80)					2.71 (1.53, 4.81)
β-Carotene, µmol/L			0.29 (0.10, 0.88)			
β-Cryptoxanthin, µmol/L			0.26 (0.07, 0.93)			
α-Tocopherol µmol/L			0.88 (0.80, 0.96)			0.88 (0.80, 0.96)
Lycopene, µmol/l				0.35 (0.17, 0.71)		
Lutein-zeaxanth., µmol/L						0.30 (0.10, 0.86)
B6 intake ≥RNI, =1	2.05 (1.19, 3.55)					
R^2 (Nagelkerke)	0.126	0.097	0.204	0.073	0.028	0.195

Multiple logistic regression models with a forward approach; values are Exp (B) = odds ratio (95% CI; all factors at $p < 0.05$, except * $p < 0.001$. Variables in the initial models included age, sex, reported malaria (in the last 3 months) or diarrhoea (in the last 4 weeks), family size (number of persons in the HH), mother's literature status (can read and write = 1), MN deficiencies (ID = 1, iron deficiency (vs. no = 0); VAD = 1, vitamin A deficiency (vs. no = 0); ZnD = 1, zinc deficiency (vs. no = 0)), serum micronutrients (e.g., β-carotene), and sufficient dietary intakes of assessed MNs (e.g., iron or vitamins ≥ RNI).

ID, VAD, ZnD, and sufficient vitamin B6 intake (intake ≥ RNI) were associated with anaemia in Chamwino; elevated acute phase proteins (CRP or AGP) were positively while serum lycopene was inversely associated with anaemia in Kilosa (Table 6). VAD and reported malaria in Chamwino and CRP/AGP in Kilosa predicted ID. In both districts, elevated CRP/AGP increased the risk whereas serum α-tocopherol reduced the risk of VAD; moreover, serum β-carotene and β-cryptoxanthin in Chamwino and higher serum lutein-zeaxanthin in Kilosa reduced the risk of VAD.

4. Discussion

The present study clearly showed that school children in the districts of Chamwino and Kilosa, Tanzania, are simultaneously affected by undernutrition; anaemia; infections, such as malaria; micronutrient deficiencies; and inadequate diets. Our results also indicate significant variations in micronutrient status and dietary habits between districts.

The overall prevalence rates of stunting (28%) and VAD (25%) were of moderate while of anaemia (>40: 22% in Chamwino and 64% in Kilosa) was of severe public health significance [12]. Stunting was in both districts highly associated with underweight and in Chamwino with younger age and VAD. Almost one in two of the stunted children in Chamwino (41/85) and Kilosa (40/102) had simultaneously underweight. This concurrent relationship shows that stunted children could simultaneously experience underweight and/or wasting (low BMI for age), and some children might experience all three forms of anthropometric failures [34], as occurred for one child in this study.

In Chamwino, the decrease in stunting prevalence with age, which is consistent with the overall decremental trend of a younger cohort of children (1–15 years) in Ethiopia [35], suggests both a higher prevalence in early childhood (<five years) and future improvement and catch-up growth. The association between VAD and stunting, in agreement with a study among preschool children in Uganda, may reflect the impact of low vitamin A status over a prolonged period due to both deficient diet and also infections (e.g., diarrhoeal diseases) on growth retardation [36]. Among the four study villages, Mhenda-Kitunduweta (Kilosa) was the village with the highest prevalence of stunting (34.8%), elevated acute phase proteins (40% with high CRP or AGP), and reported recent infection diseases (26% with diarrhoea, and 51% with malaria). The high prevalence of elevated CRP/AGP and reported malaria and diarrhoea suggest persistent and/or recurrent infections, which together with other parasitic worm diseases (i.e., helminths) lead to anaemia and micronutrient deficiencies (ID, VAD, and ZnD) and growth retardation [37].

In Tanzania and Ethiopia, stunting (and wasting) and anaemia are highly prevalent in environments that are characterized by a high prevalence of infectious diseases [5–7,38].

Apart from infections, inadequate nutrition itself with the resulting micronutrient deficiencies and anaemia can be assumed to be the decisive cause of stunting of the children in the Scale-N project. In Chamwino, multiple MN deficiencies (ID, VAD, and ZnD) and reported malaria were associated with anaemia, ID, and VAD; elevated CRP/AGP (inflammation) predicted VAD in Chamwino and anaemia, ID, and VAD in Kilosa. Further, ZnD was associated with VAD in Kilosa, while serum carotenoids and α-tocopherol reduced the risk of VAD in both districts. Overall, in Chamwino MN deficiencies whereas in Kilosa 'infections' seemed to be mainly responsible for anaemia, ID, and VAD. Further, the calculated intake of iron and vitamin A was much higher and the prevalence of anaemia, ID, and elevated CRP/AGP was much lower in Chamwino than in Kilosa. Higher iron and vitamin A intake in Chamwino was due to the fact that the main local diet consisted of wholemeal millet together with cooked indigenous dark green leafy vegetables (DGLV), as opposed to porridge of polished maize flour or rice combined with either legumes, roots, or other vegetables in Kilosa, as we recently described for the mothers in the Scale-N project [17]. The high intake of DGLV, such as the vegetable dish called 'Ilende' [23] made from a locally collected wild leafy vegetable (*Ceratotheca sesamoides*), sundried, and cooked with water and peanuts, is reflected by the significantly higher serum β-carotene and lutein-zeaxanthin than in Kilosa children. However, DGLV and other vegetables were also consumed in Kilosa, reflected in serum carotenoid concentrations [39], e.g., lutein-zeaxanthin (DGLV) or lycopene (tomato and products), and were associated with a reduced risk of anaemia and VAD. Serum lutein-zeaxanthin is apparently derived from DGLV, such as the vegetable dish 'Mlenda mgunda' (*Corchorus trilocularis*) [23], while serum lycopene indicates tomatoes as a main ingredient of various vegetable dishes, fresh vegetable salads ('kachumbari'), and cooked appetizing relishes ('chachandu').

It is surprising that the higher consumption of DGLV in Chamwino with correspondingly much higher calculated intake of RE and also slightly higher serum carotenoids did not lead to higher serum retinol (vitamin A status). This may reflect the overall limited consumption of preformed vitamin A food sources, such as meat, fish or eggs [40], as well as insufficient dietary fat intake. The food matrix, processing, and a minimum of dietary fat (3–5 g per meal) are responsible predictors for the absorption of carotenoids from vegetables [41]; the increased consumption of DGLV with oil, which improves bioavailability, was correlated with high plasma retinol among pregnant Tanzanian women [42], and bean and tomato stews with green leafy vegetable powder (GLVP) from eggplants and amaranthus leaves consumed with groundnut soup were more effective than without GLVP in reducing vitamin A deficiency and anaemia prevalence among school children (aged 4–9 years) in a Ghanaian school feeding program [43]. However, as our calculations showed, much higher amounts of MN-rich foods, including DGLV, vegetables, fruits, and meat or fish, are required to reach the RNI for minerals and vitamins according to the WHO [24]. In addition to inadequate vitamin A intake (19% overall achieved the RNI), zinc was, in particular, lacking in the diets of both districts, with only 31 of all children (4.6%) reaching the RNI of zinc. The high prevalence of ZnD (in both the diet and serum) and its association with anaemia and VAD indicate a limited diet of animal origin; poorly available MNs from plant food; and interactions among zinc, VA, and other MNs. In malnourished populations, ZnD often coexists with VAD; zinc is required in mobilizing VA within cells and from the liver through the synthesis of retinol-binding protein (RBP), while VAD may reduce the absorption and transport of zinc by altering zinc by zinc-dependent binding protein [44]. Only β-carotene supplementation along with zinc was able to improve the vitamin A status of both mothers and infants 6 months postpartum, indicating the importance of zinc in relation to VA status in a 'zinc-deficient' population [45].

Further, VAD-impaired iron metabolism in cell cultures, and supplementation with vitamin A alone, already showed a reduced risk of anaemia via higher haemoglobin and ferritin in humans with VAD [46,47]. Therefore, a population with ID, VA, or ZnD

that relies on a plant-based diet is recommended to increase the consumption of MN-dense food (β-carotene-rich fruits and vegetables) and the bioavailability of the vital nutrients, iron, β-carotene, and zinc through ideal combinations; mild cooking (with fat); and household practices, such as sprouting, fermentation, or the addition of food acidulants and spices [48–50]. A vitamin C-rich diet could increase the absorption of iron and thus reduce iron deficiency and the prevalence of anaemia. A recent controlled intervention investigating the impact of baobab fruit pulp (*Adansonia digitata* L.) on the haemoglobin and iron status of school children (aged 6–12 years) in Kenya yielded promising results [51]: the provision of a daily drink containing vitamin C-rich baobab fruit pulp (BFP) for 83 days along with standardized school meals improved haemoglobin concentrations compared to an isoenergetic drink without BFP. The authors concluded that the consumption of foods such as BFP could help improve non-heme iron absorption in populations at risk of iron deficiency, particularly in food-insecure areas where baobab is native, available, and affordable.

Furthermore, malaria and diarrhoeal infections [52,53], as reported in Chamwino and even more so in Kilosa, as well as parasitic worm infection, especially hookworms, as previously described in relation to anaemia in school children in Zanzibar and Lindi, Tanzania [8,54,55], were very likely the constant 'companions' and contributing factors to anaemia, ID, and VAD [56–59]. In addition, the lack of clean drinking water and poor sanitation and hygiene (WASH) are also very likely responsible for the high prevalence of diarrhoeal diseases [52]. Therefore, programs to reduce the prevalence of diarrhoea and parasitic infection as well as those to improve nutritional behaviour (e.g., increased consumption of fruits and vegetables) and food processing are urgently needed to reduce the high prevalence of anaemia, MN deficiencies, and finally stunting in the present study population.

Limitations of the study include the cross-section design with the use of one-time 24 h recalls, blood sampling, and anthropometric measurements, which may reflect the long-term situation to a limited extent; moreover, we did not confirm blood samples for malaria parasites and stool samples for STH infections known to be highly associated with anaemia and ID. In contrast, the strengths of this study include the large sample size with children from two different agro-ecological zones and extensive assessments that were able to indicate and confirm links among inadequate nutrient intake, MN deficiencies, and infections (through biochemical markers) and malnutrition (e.g., stunting).

5. Conclusions

In summary, our findings emphasize the high prevalence of stunting and anaemia and their associations with MN deficiencies, infections, and inadequate intakes of essential MNs (e.g., iron, vitamin A, and zinc) among Tanzanian school children of farming communities. Therefore, in a first step, the introduction of home and school gardens integrated with the provision of nutrition and health education are promising strategies to reduce the high burden of anaemia, MN deficiencies, and finally undernutrition. In parallel, programmes to reduce 'preventable' parasitic infectious diseases and to improve hygiene (sanitation) should be urgently launched.

Author Contributions: V.F.G. and W.S. were responsible for the study design; carried out the field work; and performed laboratory, data, and statistical analysis. V.F.G. wrote the manuscript, and W.S., J.K., T.J., and C.R. were responsible for reviewing and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

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CHAPTER THREE

PAPER TWO

foods

MDPI

Article

Provitamin A Carotenoids, Tocopherols, Ascorbic Acid and Minerals in Indigenous Leafy Vegetables from Tanzania

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Abstract: The essential micronutrients in indigenous leafy vegetables (ILVs) could substantially contribute to the micronutrient supply in rural communities in Tanzania, but concentrations differ between species. Provitamin A carotenoids, tocopherols, ascorbic acid, minerals, and phytate were analysed in 13 different species using HPLC-, ICP-OES, and photometric techniques. Eight of the 13 ILVs, including *Amaranthus* ssp. and *Sesamum angustifolium*, had high β-carotene concentrations (2.91–4.84 mg/100 g fresh weight), which could provide ≥50% of vitamin A's recommended nutrient intake (RNI). Six ILVs including *Cleome hirta* and *Sonchus luxurians* had high iron contents (34.5–60.4 mg/100 g, >50% RNI); *Amaranthus* ssp. represented the ILV with high calcium, magnesium and zinc contents (85%, 207% and 21% of RNI per 100 g); *Cleome hirta* and *Cleome gynandra* had high ascorbic acid contents (>15 mg/100 g, 34–35% of RNI), while *Sesamum angustifolium* was the only ILV with a high tocopherol content (7.34 mg α-TE/100 g). The highest phytate concentration was found in *Amaranthus* ssp., which could negatively affect its role as a very good source of minerals. Results indicate that the analysed ILVs could make a substantial contribution to the vitamin A and iron supply in the diets of rural Tanzanian populations.

Keywords: leafy vegetables; micronutrients; carotenoids; nutrient intake; Tanzania

1. Introduction

Indigenous leafy vegetables (ILVs) are acknowledged as part of a healthy diet due to their contribution to the dietary requirements of essential micronutrients such as vitamin A and C, iron, zinc, calcium, and magnesium [1–3]. Half of vitamin A and one-third of iron requirements in rural Tanzanian households are obtained through the consumption of indigenous leafy vegetables [4]. The typical daily main meal in Tanzania is made up of a cereal food such as rice or stiff porridge (ugali) made from maize or pearl millet flour, and is usually accompanied by a serving of cooked green leafy vegetables. These green leafy vegetables can be homegrown, bought at local markets, or harvested from the wild as indigenous species [4,5]. The term "indigenous" in this case refers to a crop species or variety genuinely native to a region, or to a crop introduced into a region where over a period of time it has evolved, although the species may not be native [4]. ILVs grow as weeds in the wild or in cultivated areas, but have also been domesticated through semi-cultivation or cultivation [6]. In addition, ILVs thrive with minimal care, are inexpensive, easily accessible, easy to cook, and could play an important role in ensuring micronutrient supply [3,7–10]. ILVs with high concentrations of provitamin A carotenoids

and iron could essentially contribute in the reduction of vitamin A and iron deficiencies in rural areas where the diet is low in animal source foods [11]. The Tanzania Demographic and Health Survey in 2010 revealed a high prevalence of iron (>29%) and vitamin A deficiency (>35%) in women aged 15–49 years [12]. The present study is part of the 'Scaling Up Nutrition' project (www.scale-n.org) which focus on the development of nutrient-sensitive strategies to improve nutrition and health status of small-scale-farmer in Dodoma and Morogoro regions; in these areas ILVs are already part of the daily diet, but detailed data on the concentrations of individual provitamin A carotenoids, vitamins and minerals in the different species are inadequate. Therefore, thirteen species of ILVs were collected from the study areas in Chamwino and Kilosa districts in order to analyse the profile of micronutrients including provitamin A carotenoids, tocopherols, ascorbic acid, and the minerals iron, zinc, magnesium, and calcium. In addition, the concentration of phytate (phytic acid-inositol hexakisphosphate) as a naturally occurring anti-nutritional factor known to affect the bioavailability of minerals was determined [10].

2. Materials and Methods

2.1. Description of the Study Area

The study was conducted in Dodoma and Morogoro regions in Tanzania. One district from each region was selected, Chamwino district in Dodoma and Kilosa district in Morogoro. Mzula and Chinoje villages in Chamwino and Tindiga and Mhenda villages in Kilosa district were selected based on factors such as geographical location, climate and consumption pattern of different indigenous vegetables (www.scale-n.org). Chamwino is located in the central plateau of Tanzania characterized by a dry Savannah and periodically semi-arid type of climate with long dry season starting late April to early December, and a short single wet season starting December to mid-April. The annual rainfall is between 350–500 mm. Kilosa District is located in eastern Tanzania characterized with sub-humid climatic condition with short rains starting in October to December and long rainfall period begin in February to May. This area has flat plains, highlands, and dry alluvial valleys with annual rainfall between 600–800 mm [13].

2.2. Sample Collection and Preparation

Thirteen different species of indigenous leafy vegetables were collected from their natural habitat during the wet season in April 2016 (Kilosa samples) and March 2017 (Chamwino samples), as shown in Table 1. Selection of the vegetable species for our research was based on the focus group discussion findings in the study areas where most women mentioned their wider consumption by the community. Identification of the vegetable samples was done by a botanist from the Department of Crop Science and Horticulture at Sokoine University of Agriculture (SUA).

The leafy edible parts of the vegetables were separated from the main plant, washed with running tap water, air-dried at room temperature at the study sites, placed in black polyethylene bags, and subsequently transported to the laboratory at SUA. The first batch of samples from Kilosa were kept from April 2016 to August 2016 in a −30 °C freezer then transported on dry ice to the University of Hohenheim, Stuttgart in Germany; stored at −80 °C, freeze-dried, and analysed in September 2016. The second batch of samples from Chamwino were kept for two days in a −30 °C freezer, then immediately transported on dry ice to the University of Hohenheim, Stuttgart in Germany; stored at −80 °C for one week, then freeze-dried and analysed. The individual ILV species from both sample batches, were freeze-dried for 24 h (to constant weight), protected from light (using aluminium foil) using a Telstar freeze drier (2014 model LyoQuest; Telstar, Spain), ground in a mortar to a fine homogenous powder and stored in airtight containers protected from light; aliquots of the (freeze-dried) powder were finally weighed as samples for individual analyses on micronutrients and phytate contents. Details regarding botanical names, local names, villages of collection, and the conversion factors of the analysed ILVs are given in Table 1. Samples were weighed before and after

freeze-drying and the respective factor for converting results per freeze-dried weight (FDW) to fresh weight (FW) was calculated according to the following Formula (1):

$$\text{Conversion factor} = (b - a)/(c - a) \quad (1)$$

where a = weight of empty bottle; b = weight of the sample with the bottle before freeze-drying; and c = weight of the sample with the bottle after freeze-drying. Details of weight (or moisture) loss due to freeze-drying of the different ILV species are given in Table A1 (Appendix A).

Table 1. Vegetable samples origin and their conversion factors.

Sample Origin-District	Botanical Name	Local Names	Sample Origin-Village	Conversion Factor *
Chamwino	*Ceratotheca sesamoides*	Ilende	Chinoje	4.34
	Vigna unguiculata	Safwe	Chinoje	5.73
	Cleome gynandra	Mzimwe	Mzula	6.97
	Cleome hirta	Mhilile	Mzula	7.46
	Ipomoea obscura	Sagula sagula	Mzula	11.55
	Ipomoea pandurata	Chiwandagulu	Mzula	8.54
Kilosa	*Bidens pilosa*	Mashona nguo	Mhenda	6.42
	Justicia heterocarpa	Mwidu	Mhenda	5.22
	Sesamum angustifolium	Mlenda ufuta	Mhenda	3.99
	Amaranthus spinosus	Mchicha chawa	Tindiga	6.56
	Amaranthus spp.	Mchicha bwasi	Tindiga	2.42
	Corchorus trilocularis	Mlenda mgunda	Tindiga	5.65
	Sonchus luxurians	Sunga	Tindiga	8.19

* Conversion factor = FW/FDW, fresh weight/freeze-dried weight.

2.3. Analytical Methods

All determinations were done in freeze-dried samples (powder). Carotenoids, tocopherols, and ascorbic acid (AA) were analysed using High-Performance Liquid Chromatography (HPLC). The minerals iron, calcium, magnesium, zinc, and phosphorus were determined using Inductively Coupled Plasma-Optical Emission Spectrophotometry (ICP-OES), while phytic acid content was analysed after clean-up using a photometric method. Micronutrient and phytate contents in homogenized freeze-dried ILV samples were determined as milligrams per 100 g dry matter (mg/100 g) and finally converted and presented per fresh weight using the dry-to-fresh conversion factor (Table 1). The conversion from freeze-dried weight to fresh weight was calculated according to the following Formula (2):

$$\text{Values per fresh weight} = (\text{Values per freeze} - \text{dried weight})/\text{Conversion factor} \quad (2)$$

where (values per freeze − dried weight) are the concentrations per 100 g freeze-dried weight of respective micronutrients and phytic acid content measured by the HPLC, ICP-OES, and photometric method. Finally, all results (in the tables) are given in mg or µg/100 g of estimated fresh weight.

2.3.1. Determination of Carotenoids and Tocopherols

Carotenoids and tocopherols were extracted and analysed via HPLC, as previously described by Stuetz et al. [14]. Briefly, 100 mg of freeze-dried ILV samples were saponified in screw-capped glass tubes under stirring in a light protected water bath (2.5 h, at 38 °C) by adding 500 µL of KOH (50% w/w), and 1 mL ethanol containing β-apo-8′-carotenal methyloxime (1 µL/mL) as an internal standard. After saponification, 2 mL of saline solution (15% w/V) was added, then samples were neutralised with 500 µL of glacial acetic acid. The fat-soluble components were extracted with hexane (2 × 1 mL), and combined fractions were evaporated in a rotational vacuum evaporator (RVC 2-33 IR, Christ, Osterode am Harz, Germany). The residue was redissolved in 70 µL ethanol (>96%) and

210 µL acetonitrile in order to be analyzed using RP-HPLC, UV-vis (450 nm for carotenoids) and fluorescence detection (Excitation set at 298 nm and Emission set at 328 nm for α-/γ-tocopherol). All reagents and solvents were of analytical and (ultra) gradient HPLC grade. N-hexane, 1, 4-dioxane, potassium hydroxide solution (50%), acetic acid (100%), and ethanol were from Carl Roth GmbH + Co. KG (Karlsruhe, Germany), while methanol and acetonitrile were from J.T. Baker (Deventer, The Netherlands).

Recommended daily safe intake of 500 µg retinol equivalents (RE) for vitamin A intake and daily acceptable intakes of 7.5 mg of α-tocopherol equivalents (α-TE) for vitamin E intake among females aged 19–50 years were used to estimate the contribution of ILVs [15]. Conversion factors of 12:1 for β-carotene and 24:1 for other β-cryptoxanthin and α-carotene were applied for the calculation of RE, while α-TE were calculated using conversion factors of 1:1 for α-tocopherol and 10:1 for γ-tocopherol [15].

2.3.2. Determination of Ascorbic Acid (AA)

Ascorbic acid in 25 mg of freeze-dried samples were extracted with a mixture of 550 µL of freshly prepared 10% (*w*/*w*) meta-phosphoric acid (MPA) and 50 µL of 20% (*w*/*w*) tris-(2-carboxyethyl)-phosphine (TCEP, $C_9H_{15}O_6P{\cdot}HCl$) solution as the reduction agent; samples were incubated for 5 min on ice (in the dark, protected from light), then vortex-mixed for 3 min, and centrifuged at 17,000 × *g* for 10 min; 20 µL of supernatants were analysed on AA content using a Shimadzu Prominence HPLC, a Reprosil-Pur 120 C18 AQ analytical column (5 µm, 250 × 4.6 mm, Dr. Maisch GmbH, Ammerbuch, Germany), sodium dihydrogen-phosphate buffer (0.1 M NaH_2PO_4 x $3H_2O$, set to pH 2.5) as a mobile phase at a flow rate of 1 mL/min and an UV-Vis detector (SPD-20A, Shimadzu, Kyoto, Japan) set at 245 nm [16]. TCEP, MPA, and reagents were from Carl Roth GmbH + Co. KG, Karlsruhe, Germany. The recommended nutrient intake (RNI) was calculated based on 45 mg/day recommendation of Vitamin C for women 19–50 years [15].

2.3.3. Determination of Minerals

Iron (Fe), zinc (Zn), calcium (Ca), and magnesium (Mg) contents in 250 mg of samples were determined by ICP-OES after microwave-heated nitric acid digestion using an ultraclave, as previously described [17]. The RNI were calculated based on, iron (58.8 mg/day, 5% bioavailability); zinc (9.8 mg/day, low bioavailability); calcium (1000 mg/day) and magnesium (220 mg/day) for women 19–50 years [15].

2.3.4. Determination of Phytate (and Its Ratio to Analysed Minerals)

Phytate (inositol hexakisphosphate, IP6) was quantitatively measured by photometry [18] after extraction and clean up using solid-phase extraction (SPE) and anion-exchange purification [19]. In brief, 150 mg of samples were extracted using 0.1 M HCl (1.5 mL) and sonication for 30 min. Following samples were centrifuged twice (60 min at 13,300 rpm) and clear supernatants of ILV extracts (500 µL) were diluted (1:20) and adjusted to pH 6 in order to be transferred to SPE glass cartridges filled with 500 mg of anion exchange resin (AG1-X8 resin, Bio-Rad laboratories, Inc., Hercules, CA, USA). Samples were washed (0.1 M NaCl to remove free phosphate) and phytates were eluted with 0.7 M NaCl. Samples were adjusted to pH 3, mixed with Wade reagent (0.06% $FeCl_3{\cdot}6H_2O$ + 0.6% sulfosalicylic acid) and absorbance was measured at 490 nm. Phytate (phytic acid) standards in concentrations from 25 µg/mL to 200 µg/mL and blanks (Wade reagent) were used for calibration. To predict the bioavailability of calcium, iron and zinc; molar weights (phytate: 660 g/mol; Ca: 40 g/mol; Fe: 56 g/mol; and Zn: 65 g/mol) were used to calculate the (molar) ratio of phytate to individual mineral concentrations of the ILVs [20].

2.4. Statistical Analysis

Results are concentrations per wet weight (fresh weight) and described as means and standard deviations. Data used for provitamin A carotenoids, ascorbic acid and tocopherols were means of triplicate ($n = 3$) determinations while for minerals and phytate were of duplicate ($n = 2$) determinations. Multiple comparisons between ILVs were performed by one-way analysis of variance (ANOVA) and post-hoc Tukey's HSD (Honestly Significant Difference) test; statistical significance was considered at a p value < 0.05. All statistical analyses were carried out using IBM SPSS software (Version 23, IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Provitamin A Carotenoids Contents

The provitamin A carotenoids contents and the contribution of different vegetable species to the RNI of vitamin A (retinol equivalents-RE) are presented in Table 2. Table 2 shows a range of β-carotene contents between 1.01 and 4.84 mg/100 g. *Amaranthus* spp. (4.84 mg/100 g) had a high β-carotene content compared to other ILVs. *Sesamum angustifolium* (4.06 mg/100 g), *Corchorus trilocularis* (4.04 mg/100 g), and *Justicia heterocarpa* (3.84 mg/100 g) were also rich in β-carotene (Table 2). In comparison to other analysed ILVs, *Sesamum angustifolium* (0.18 mg/100 g) and *Justicia heterocarpa* (0.95 mg/100 g) had the highest content of β-cryptoxanthin and α-carotene, respectively.

Table 2. Provitamin A carotenoids contents (mg/100 g fresh weight) of ILVs *.

Botanical Names	β-Cryptoxanthin	α-Carotene	β-Carotene	RE [1] (µg/100 g)	% of RNI [2]
Amaranthus spinosus	$0.04 \pm 0.01^{f,g}$	$0.26 \pm 0.01^{b,c}$	$3.65 \pm 0.17^{a,b,c}$	$317 \pm 14^{a,b,c}$	63
Amaranthus spp.	$0.13 \pm 0.01^{b,c,d}$	0.32 ± 0.01^{b}	4.84 ± 0.16^{a}	422 ± 13^{a}	84
Bidens pilosa	$0.05 \pm 0.01^{f,g}$	$0.07 \pm 0.01^{c,d}$	$2.07 \pm 0.07^{c,d,e}$	$178 \pm 6^{c,d,e}$	36
Cleome gynandra	$0.13 \pm 0.02^{b,c,d}$	0.05 ± 0.02^{d}	$2.91 \pm 0.41^{b,c,d}$	$250 \pm 36^{b,c,d}$	50
Cleome hirta	$0.06 \pm 0.03^{e,f,g}$	0.01 ± 0.01^{d}	$2.75 \pm 0.20^{b,c,d}$	$232 \pm 17^{b,c,d}$	46
Corchorus trilocularis	$0.11 \pm 0.01^{c,d,e}$	$0.09 \pm 0.01^{c,d}$	$4.04 \pm 0.57^{a,b}$	$345 \pm 48^{a,b}$	69
Ceratotheca sesamoides	$0.14 \pm 0.01^{b,c}$	$0.07 \pm 0.02^{c,d}$	$1.96 \pm 0.35^{d,e}$	$172 \pm 30^{d,e}$	34
Ipomoea obscura	$0.09 \pm 0.01^{c,d,e,f}$	$0.07 \pm 0.01^{c,d}$	1.01 ± 0.04^{e}	90 ± 3^{e}	18
Ipomoea pandurata	$0.05 \pm 0.03^{f,g}$	$0.16 \pm 0.01^{b,c,d}$	$2.99 \pm 0.15^{b,c,d}$	$258 \pm 13^{b,c,d}$	52
Justicia heterocarpa	$0.07 \pm 0.01^{e,f,g}$	0.95 ± 0.27^{a}	$3.84 \pm 1.45^{a,b}$	$363 \pm 132^{a,b}$	73
Sesamum angustifolium	$0.18 \pm 0.01^{a,b}$	$0.06 \pm 0.01^{c,d}$	$4.06 \pm 0.39^{a,b}$	$349 \pm 33^{a,b}$	70
Sonchus luxurians	0.03 ± 0.01^{g}	0.03 ± 0.01^{d}	$2.15 \pm 0.03^{c,d,e}$	$181 \pm 2^{c,d,e}$	36
Vigna unguiculata	$0.08 \pm 0.01^{d,e,f,g}$	$0.12 \pm 0.05^{b,c,d}$	$2.96 \pm 1.26^{b,c,d}$	$255 \pm 107^{b,c,d}$	51

* ILVs, indigenous leafy vegetables: values are means ± SD; values within a column not sharing a common superscript letter (a,b,c,d,e,f,g) are significantly different (Tukey ≤ 0.05). [1] RE, retinol equivalents (sum of RE), for which 1 RE = 12 µg β-carotene or 24 µg α-carotene or 24 µg β-cryptoxanthin. [2] RNI, recommended nutrient intakes by WHO/FAO [15]; values as (microgram) for female adults (19–50 years).

β-Carotene contents of *Cleome gynandra*, *Sonchus luxurians*, and *Amaranthus* spp., from this study, were in agreement with other reported values of ILVs in Tanzania [4,21]. Limited results are available for β-cryptoxanthin and α-carotene comparison. A study on ILVs from Cameroon [22], reported contents (per dry weight) of β-cryptoxanthin (0.21 ± 0.03 mg/100 g) and α-carotene (0.21 ± 0.05 mg/100 g) in *Ceratotheca sesamoides*. However, a strong comparison cannot be made between the reported contents and data from the present study, as different drying techniques were used: Sun drying in the previous work versus freeze-drying in the present study.

The stability and retention of carotenoids in food are influenced by various factors including their chemical nature, storage time, storage conditions, cooking, and preparation methods [2,23]. Indigenous leafy vegetables in Tanzania are mostly prepared by steaming, boiling, or stir-frying together with other vegetables such as tomatoes and onions. Steaming and stir-frying with oil are mentioned as desirable methods for preparing ILVs in view of carotenoids retention and bioavailability [2,23,24]. Due to different plant matrices, the bioavailability of carotenoids from orange-pigmented fruits was superior

to that of dark-green leafy vegetables, as reported by De Pee et al. [25]. However, the bioavailability of β-carotene and other carotenoids can be enhanced through food processing (cooking, homogenisation) and fat in the diet [26–28]. Moderate cooking and/or mechanical homogenisation, which destroys the cell walls, increase the release of carotenoids, while a sufficient amount of dietary fat (3–5 g per meal), especially if rich in unsaturated fatty acids, is essential for maximal carotenoid absorption from vegetables [27,28]. Results in the present study indicate *Amaranthus* spp., *Justicia heterocarpa*, *Sesamum angustifolium*, *Corchorus trilocularis*, *Amaranthus spinosus*, *Ipomoea pandurata*, *Vigna unguiculata* and *Cleome gynandra* as rich sources of vitamin A since the consumption of 100 g of these vegetable species can provide between 50% to 84% of the RNI for retinol equivalents (RE). A study conducted in central and North-Eastern parts of Tanzania [29] reported an average per capita vegetable intake of 207 g per day, suggesting a contribution of 104 to 174 percent of the RNI for retinol equivalents (RE), and thus underlining an important contribution of these ILVs to ensuring vitamin A intake by the population in the study areas.

3.2. Tocopherols and Ascorbic Acid Contents

Low contents of γ- and α-tocopherol were observed in almost all analysed samples; *Sesamum angustifolium* being the exception with a high concentration of α-tocopherol (7.29 mg/100 g) (Table 3). In comparison to other ILVs, *Amaranthus* spp. exhibited the highest γ-tocopherol content (0.67 mg/100 g) followed by *Sesamum angustifolium* (0.54 mg/100 g). A 100 g portion of *Sesamum angustifolium* with the highest level of α-tocopherol equivalents (α-TEs) could provide 98% of the acceptable intake for vitamin E (Table 3). This could be explained by the presence of immature seeds that were part of the leaves in the sample; further research and analysis on separated leaves and seeds are required to describe and confirm *Sesamum angustifolium* as an excellent source for vitamin E. Until now, little information is available on the tocopherol contents of *Sesamum angustifolium* leaves. One previous study on *Sesamum angustifolium* seeds [30], reported total tocopherol contents of approximately 760 mg/kg oil, consisting γ- and δ-tocopherol, but no α-tocopherol. Studies done on the leaf of the sesame species *Sesamum indicum* reported the presence of α-tocopherol content (0.55 mg/100 g edible portion) but no detection of γ-tocopherol [31]. Vitamin E is the major lipid-soluble antioxidant that provides protection against lipid peroxidation and protects components of cell membranes from oxidation by free radicals, thus preventing or delaying the onset of chronic diseases associated with reactive oxygen species molecules [15,32]. The richest sources of tocopherols include nuts, seeds, and vegetable oils [32]. Results from the present study suggest further research into the contribution of *Sesamum angustifolium* as a source of tocopherols in the diet.

The ascorbic acid contents of 13 analysed ILVs ranged between 0.08 mg/100 g for *Justicia heterocarpa*, to 15.6 mg/100 g for *Cleome hirta* (Table 3). In previous studies a vitamin C content of 2 mg/100 g in *Amaranthus* spp., 9 mg/100 g in *Vigna unguiculata* and 2 mg/100 g in *Cleome gynandra* [3] was reported, which by contrast shows higher contents for *Amaranthus* spp and *Vigna unguiculata* but lower for *Cleome gynandra* (Table 3). Ascorbic acid values for *Cleome gynandra* are in agreement with that reported in other studies [1,33]. Variations in the contents of ascorbic acid in ILVs are known to be related to various factors such as plant variety or species, maturity stage, storage time and high temperature [33,34]. Vitamin C is a water-soluble antioxidant required for normal metabolic functions of the body and plays an important role as an enhancer of dietary Fe absorption [35,36]. Since non-heme Fe is the only Fe compound in plants, its absorption is said to be improved by dietary constituents such as ascorbic acid and other organic acids [37]. Five of the analysed ILVs could contribute more than 10% of the RNI by 100 g fresh weight, with *Cleome hirta* and *Cleome gynandra* reaching 35% and 34% of the RNI, and thus the reported average per capita vegetable intake of 207 g per day [29], would provide between 71 and 72 percent of the RNI for vitamin C (Table 3). Since longer storage times and higher temperatures reduce the ascorbic acid contents, a significant ascorbic acid supply through *Cleome hirta* and *Cleome gynandra* can only be achieved when these leaves are utilized immediately after harvesting. Additionally, shorter cooking times, with low

water content, as well as the consumption of the cooking water are also recommended as measures to ensure ascorbic acid retention and intake [38].

Table 3. Tocopherols and ascorbic acid contents (mg/100 g fresh weight) in ILVs *.

Botanical Names	γ-Tocopherol	α-Tocopherol	α-TE [1]	% AI [2]	Ascorbic Acid	% RNI [3]
Amaranthus spinosus	0.01 ± 0.01 ^{c}	0.01 ± 0.01 ^{b}	0.01 ± 0.01 ^{b}	0	0.30 ± 0.02 ^{g}	1
Amaranthus spp.	0.67 ± 0.05 ^{a}	0.01 ± 0.01 ^{b}	0.07± 0.01 ^{b}	1	0.48 ± 0.01 ^{f,g}	1
Bidens pilosa	0.02 ± 0.01 ^{c}	0.03 ± 0.02 ^{b}	0.03 ± 0.02 ^{b}	0	1.20 ± 0.05 ^{f,g}	3
Cleome gynandra	0.01 ± 0.01 ^{c}	0.02 ± 0.01 ^{b}	0.02 ± 0.01 ^{b}	0	15.44 ± 0.91^{a}	34
Cleome hirta	0.01 ± 0.01 ^{c}	0.01 ± 0.01 ^{b}	0.01 ± 0.01 ^{b}	0	15.60 ± 0.24^{a}	35
Corchorus trilocularis	0.04 ± 0.06 ^{c}	0.04 ± 0.03 ^{b}	0.04 ± 0.04 ^{b}	1	0.25 ± 0.01^{g}	1
Ceratotheca sesamoides	0.02 ± 0.01 ^{c}	0.03 ± 0.01 ^{b}	0.03 ± 0.01 ^{b}	0	6.48 ± 0.07^{d}	14
Ipomoea obscura	0.01 ± 0.01 ^{c}	0.01 ± 0.01 ^{b}	0.01 ± 0.01 ^{b}	0	1.60 ± 0.15 ^{f}	4
Ipomoea pandurata	0.04 ± 0.01 ^{c}	0.07 ± 0.01 ^{b}	0.07 ± 0.01^{b}	1	11.28 ± 0.13 ^{b}	25
Justicia heterocarpa	0.03 ± 0.01^{c}	0.02 ± 0.01 ^{b}	0.02 ± 0.01 ^{b}	0	0.08 ± 0.01^{g}	0
Sesamum angustifolium	0.54 ± 0.06 ^{b}	7.29 ± 0.75 ^{a}	7.34 ± 0.75 ^{a}	98	4.09 ± 0.22 ^{e}	9
Sonchus luxurians	0.01 ± 0.01 ^{c}	0.01 ± 0.01 ^{b}	0.01± 0.01 ^{b}	0	0.68 ± 0.03 ^{f,g}	2
Vigna unguiculata	0.04 ± 0.01 ^{c}	0.02 ± 0.01 ^{b}	0.02 ± 0.01 ^{b}	0	8.96 ± 0.01 ^{c}	20

* ILVs, indigenous leafy vegetables: values are means ± SD; values within a column not sharing a common superscript letter (a,b,c,d,e,f,g) are significantly different (Tukey ≤ 0.05). [1] α-TEs, α-tocopherol equivalents (1 mg α-TE = 1 mg α-tocopherol or 10 mg γ-tocopherol). [2] AI, acceptable intake [15]. [3] RNI, recommended nutrient intakes by WHO/FAO [15]; values in (milligram) for female adults (19–50 years).

3.3. Mineral Contents

The mean values of the vegetable mineral contents and their contribution to the RNI are presented in Tables 4 and 5. Considerable amounts of Fe (2.4–60.4 mg/100 g) Zn (0.37–2.05 mg/100 g), Ca (100–853.7 mg/100 g), and Mg (37.7–455.8 mg/100 g) were found in the analysed ILV species. The ILVs with highest Fe contents were *Amaranthus* spp. (60.4 mg/100 g), *Cleome hirta* (56.4 mg/100 g), *Corchorus trilocularis* (43.5 mg/100 g), *Sonchus luxurians* (42.2 mg/100 g), *Cleome gynandra* (39.0 mg/100 g), and *Ceratotheca sesamoides* (34.5 mg/100 g). *Amaranthus* spp. also contained the highest amount of Zn (2.05 mg/100 g) in comparison to other ILVs (Table 4). Previous studies in Tanzania [4,39] reported Fe and Zn contents in *Amaranthus* spp., *Bidens pilosa*, *Sonchus luxurians*, *Chorchorus trilocularis*, and *Vigna unguiculata* in agreement with the ones in the present study. In a review on *Bidens pilosa* [2], Fe contents ranging between 2.0 to 6.0 mg/100 g fresh weight, Zn between 0.9 to 2.6 mg/100 g fresh weight, and Ca between 162 to 340 mg/100 g fresh weight were similar with contents reported in the present study. Other researchers [1,40,41], however, reported lower Fe concentrations (2.48–14.4 mg/100) in *Bidens pilosa*, *Cleome gynandra*, *Amaranthus spinosus*, and *Corchorus trilocularis* compared to those reported in the present study. The Zn content for *Bidens pilosa* was in agreement with the one reported by Reference [41].

Iron plays numerous biochemical roles in the body including the formation of red blood cells [9]. Limited supply of Fe in most usual African diets is due to low intake of animal-based foods [42]. ILVs such as *Amaranthus* spp., *Cleome hirta*, *Corchorus trilocularis* and *Sonchus luxurians* with high Fe content which could contribute 103%, 96%, 74%, and 72% to the RNI per 100 g, respectively, can be recommended to alleviate iron deficiency anaemia in communities with limited access to animal food sources.

Zinc is important in gastrointestinal and immune functions, energy metabolism and as a co-enzyme in numerous biochemical reactions in the body [2,9]. Low contents of Zn were recorded in most of the analysed ILVs (Table 4). In comparison to other analysed ILV species, *Amaranthus* spp. had a high Zn content (2.05 mg/100 g), which could contribute to 21% to the RNI (Table 4). Similar results were reported in studies conducted in South Africa on *Amaranthus spinosus*, *Bidens pilosa*, *Cleome gynandra*, and *Vigna unguiculata* [3,41]. Overall, these results show that the analysed ILVs can contribute to a lesser extent to Zn nutrition in comparison to Fe.

Table 4. Iron and zinc contents (mg/100 g fresh weight) of ILVs *.

Botanical Names	Iron	% of RNI [1]	Zinc	% of RNI [1]
Amaranthus spinosus	19.8 ± 1.7 [d]	34	0.82 ± 0.01 [b]	8
Amaranthus spp.	60.4 ± 3.6 [a]	103	2.05 ± 0.01 [a]	21
Bidens pilosa	3.3 ± 0.2 [g]	6	0.86 ± 0.01 [b]	9
Cleome gynandra	39.0 ± 2.7 [b,c]	66	0.46 ± 0.01 [e]	5
Cleome hirta	56.4 ± 2.5 [a]	96	0.46 ± 0.01 [e]	5
Corchorus trilocularis	43.5 ± 1.1[b]	74	0.46 ± 0.01[e]	5
Ceratotheca sesamoides	34.5 ± 0.7 [c]	59	0.67 ± 0.01 [d]	7
Ipomoea obscura	19.6 ± 0.4 [d]	33	0.38 ± 0.01[f]	4
Ipomoea pandurata	10.3 ± 0.0 [e,f]	18	0.37 ± 0.02 [f]	4
Justicia heterocarpa	10.5 ± 0.5 [e,f]	18	0.75 ± 0.04 [c]	8
Sesamum angustifolium	2.4 ± 0.3 [g]	4	0.72 ± 0.01 [c]	7
Sonchus luxurians	42.2 ± 0.7 [b]	72	0.40 ± 0.01 [f]	4
Vigna unguiculata	5.1 ± 0.1 [f,g]	9	0.41 ± 0.01 [f]	4

* ILVs, indigenous leafy vegetables: values are means ± SD; values within a column not sharing a common superscript letter (a,b,c,d,e,f,g) are significantly different (Tukey ≤ 0.05). [1] RNI, recommended nutrient intakes by WHO/FAO [15]; values as (milligram) for female adults (19–50 years).

Table 5. Calcium and magnesium (mg/100 g fresh weight) of ILVs *.

Botanical Names	Calcium	% of RNI [1]	Magnesium	% of RNI [1]
Amaranthus spinosus	289.1 ± 9.2 [c,d]	29	202.9 ± 0.3 [b]	92
Amaranthus spp.	853.7 ± 24.9 [a]	85	455.8 ± 0.1 [a]	207
Bidens pilosa	163.1 ± 1.1 [g,h]	16	60.0 ± 0.2 [i]	27
Cleome gynandra	260.1 ± 2.8 [d,e]	26	70.1 ± 0.1 [fg]	32
Cleome hirta	310.5 ± 1.3 [c]	31	44.8 ± 0.0 [j]	20
Corchorus trilocularis	167.3 ± 2.9 [g]	17	62.0 ± 0.4 [i]	28
Ceratotheca sesamoides	248.8 ± 3.1 [e,f]	25	129.4 ± 1.8 [c]	59
Ipomoea obscura	100.0 ± 1.7 [i]	10	37.7 ± 0.5 [l]	17
Ipomoea pandurata	143.3 ± 1.0 [g,h]	14	67.2 ± 0.6 [h]	31
Justicia heterocarpa	478.6 ± 3.3 [b]	48	79.7 ± 0.1 [e]	36
Sesamum angustifolium	168.4 ± 5.1 [g]	17	83.7 ± 1.4 [d]	38
Sonchus luxurians	135.3 ± 1.2 [h]	14	41.1 ± 0.3 [k]	19
Vigna unguiculata	274.2 ± 4.4 [d,e]	27	70.8 ± 1.0 [f]	32

* ILVs, indigenous leafy vegetables: values are means ± SD; values within a column not sharing a common superscript letter (a,b,c,d,e,f,g,h,i,j,k,l) are significantly different (Tukey ≤0.05). [1] RNI, recommended nutrient intakes by WHO/FAO [15]; values as (milligram) for female adults (19–50 years).

The range of Ca in the ILVs was between 100 mg/100 g in *Ipomoea obscura* and 853.7 mg/100 g *Amaranthus* spp., while for Mg it was between 37.7 mg/100 g in *Ipomoea obscura* and 455.8 mg/100 g in *Amaranthus* spp. (Table 5). The Ca content was higher in *Amaranthus* spp., followed by *Justicia heterocarpa*: an estimated consumption of 100 g could contribute 85% (*Amaranthus* spp.) and 48% (*Justicia heterocarpa*) of the RNI for Ca. In comparison to other analysed ILVs, the Mg content in *Amaranthus* spp. (455.8 mg/100 g), and *Amaranthus spinosus* (202.9 mg/100 g) was high, contributing a possible 207% and 92% of the RNI, respectively. Ca contents of *Bidens pilosa* and *Cleome gynandra* were in similar ranges of those reported by [3,41]. The values of magnesium in *Amaranthus spinosus* and *Cleome gynandra* were in agreement with the ones reported by [41]. Ca and Mg play important roles in development and maintenance of healthy bones and muscles [9]. A 100 g portion of the analysed ILVs did not meet the RNI for calcium but *Amaranthus* spp. and *Justicia heterocarpa* can be regarded as valuable Ca sources with possible contributions of 85% and 48% to the RNI (Table 5). *Amaranthus* spp. was the ILV with a high Mg content, reaching 2-fold the RNI per 100 g fresh weight. The bioavailability of Ca and Mg is dependent on the age and sex of an individual, fat content in the diet and the presence of antinutrients [2]. Therefore, *Amaranthus* spp., *Amaranthus spinosus*, and *Ceratotheca sesamoides* could

potentially contribute towards the dietary requirements of these two minerals when cooking methods such as boiling, steaming, and stir-frying with oil are applied [7,9,24,43].

The quantitative analysis on minerals in ILVs revealed considerable high contents of Fe, Ca, and Mg in certain ILV species and therefore an important contribution to recommended daily mineral intakes. Most notable are the high concentrations of Fe, Ca and Mg in *Amaranthus* spp. compared to other ILVs. *Cleome gynandra* and *Cleome hirta* could serve as important ILVs in iron nutrition due to their appreciable contents of ascorbic acid that enhances Fe absorption. By contrast, the analysed ILVs could not serve as very good sources of Zn, since only 21% of the RNI was reached by the highest Zn content in *Amaranthus* spp. (Table 5). However, in comparison to the Zn contents of boiled rice (0.5 mg/100 g) and maize stiff porridge (0.6 mg/100 g), which are important staple dishes in Tanzania [44], *Amaranthus* spp. has more zinc content and this suggests that its integration could improve Zn content in the consumed diets.

3.4. Phytate Content

The phytate contents and molar ratios along with the suggested critical values for estimating the effect of phytate on the bioavailability of Fe, Zn, and Ca are presented in Table 6. *Amaranthus* spp. (739 mg/100 g) and *Amaranthus spinosus* (334 mg/100 g) had the highest phytate contents compared to other ILVs (Table 6). The calculated phytate: Iron molar ratios for six ILV samples (Table 6) were above the suggested critical level (>1) indicating poor iron bioavailability [45,46]. In this context, *Cleome gynandra*, *Cleome hirta*, *Corchorus trilocularis*, *Sonchus luxurians*, and *Ceratotheca sesamoides* with phytate:iron molar ratio <l are better sources of bioavailable iron (Table 6). The phytate: zinc molar ratios of *Ceratotheca sesamoides*, *Sonchus luxurians*, *Bidens pilosa*, *Cleome hirta* and *Justicia heterocarpa* were below the suggested critical level of 15, above which zinc bioavailability is seriously impaired [46]. *Amaranthus* spp. had high contents of Ca, Fe, and Zn (Tables 4–6), a low phytate:calcium (0.05), a still acceptable phytate:iron (1.04), but an unfortunately high phytate:zinc molar ratio (35.71). However, in consideration of the applied RNI for low zinc and 5% iron bioavailability, *Amaranthus* spp. can serve as an important source of Fe and Zn in the diet. The phytate: Calcium molar ratios in all the leafy vegetable samples ranged between 0.01–0.07, which is well below the critical level of 0.24, above which it is said that calcium bioavailability is impaired [46]. This suggests that the phytate contents of the analysed ILVs will not have a substantial impact on the calcium bioavailability.

Table 6. Phytate (mg/100 g fresh weight) and its ratios to minerals in ILVs *.

Botanical Names	Phytate [1]	Phytate:iron	Phytate:zinc	Phytate:calcium
Amaranthus spinosus	334 ± 61 [b]	1.43	40.35	0.07
Amaranthus spp.	739 ± 74 [a]	1.04	35.71	0.05
Bidens pilosa	119 ± 21 [c]	3.06	13.71	0.04
Cleome gynandra	120 ± 6 [c]	0.26	25.84	0.03
Cleome hirta	55 ± 16 [c]	0.08	11.84	0.01
Corchorus trilocularis	158 ± 3 [c]	0.31	34.02	0.06
Ceratotheca sesamoides	57 ± 28 [c]	0.14	8.43	0.01
Ipomoea obscura	68 ± 18 [c]	0.29	17.73	0.04
Ipomoea pandurata	104 ± 27 [c]	0.86	27.84	0.04
Justicia heterocarpa	103 ± 8 [c]	0.83	13.60	0.01
Sesamum angustifolium	146 ± 30 [c]	5.16	20.09	0.05
Sonchus luxurians	44 ± 11 [c]	0.09	10.90	0.02
Vigna unguiculata	109 ± 26 [c]	1.81	26.33	0.02

* ILVs, indigenous leafy vegetables: [1] values are means ± SD; values within a column not sharing a common superscript letter (a,b,c) are significantly different (Tukey $\leq$ 0.05); Critical values of molar ratios predicting the inhibitory effect of phytate on Fe, Zn, and Ca: phytate:iron >1, phytate:zinc >15, phytate:calcium >0.24 [46].

Phytate, which is the major storage form of phosphorus in plants [2] has the ability to form chelates with di-and tri-valent metallic ions such as Zn, Fe, Mg, and Ca to form poorly soluble compounds

that are not readily absorbed from the gastrointestinal tract, thus decreasing their bioavailability [47]. Previous studies reported that traditional food preparation and cooking methods (fermentation, boiling, or frying) can significantly reduce phytate content in vegetables [7,9,10,43]. Therefore, the diminishing effect on phytate content by suggested cooking methods can even increase the contribution of ILVs as important sources of Fe, Zn, and Ca.

4. Conclusions

The analysed indigenous leafy vegetables (ILVs) contain substantial amount of provitamin A carotenoids, minerals, but also the anti-nutritional factor phytate. There are differences in concentrations of essential micronutrients between ILV species and thus diverse consumption is beneficial in terms of micronutrient supply. The remarkable contents of iron and β-carotene revealed in the ILVs can serve an important role to safeguard iron and vitamin A supply in the study area. In rural settings where households rely on the consumption of ILVs improved preparation and processing methods to ensure carotenoids retention and reduction of phytate would be further measures to ensure vitamin A and iron supply in the population.

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

Appendix A

Table A1. Weights of ILVs before and after freeze drying in grams *.

Botanical Names	Fresh Weight [1] (gram)	Dried Weight [2] (gram)	Moisture Loss [3] (gram)	Moisture Loss [4] (%)
Amaranthus spinosus	10.048	1.531	8.517	84.76
Amaranthus spp.	38.660	15.99	22.67	58.64
Bidens pilosa	10.216	1.591	8.625	84.43
Cleome gynandra	45.380	6.510	38.87	85.65
Cleome hirta	68.430	9.170	59.26	86.60
Corchorus trilocularis	12.744	2.257	10.487	82.29
Ceratotheca sesamoides	205.99	47.47	158.52	76.96
Ipomoea obscura	86.380	7.480	78.900	91.34
Ipomoea pandurata	166.59	19.50	147.09	88.29
Justicia heterocarpa	12.937	2.479	10.458	80.84
Sesamum angustifolium	9.179	2.298	6.881	74.96
Sonchus luxurians	11.903	1.453	10.450	87.79
Vigna unguiculata	207.39	36.21	171.18	82.54

* ILVs, indigenous leafy vegetables: [1] Fresh weight = weight before freeze drying; [2] Dried weight = weight after freeze drying; [3] Moisture loss = fresh weight − dried weight; [4] Moisture loss = Moisture loss /Fresh weight × 100.

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CHAPTER FOUR

PAPER THREE

4.0 ADDRESSING ANAEMIA AND MICRONUTRIENT DEFICIENCIES AMONG SCHOOL CHILDREN IN RURAL TANZANIA: RESULTS OF AN INTEGRATED HOME GARDENING INTERVENTION

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Abstract

Anaemia and micronutrient (MN) deficiencies are common among school children in Tanzania. However, the influence of food-based interventions on school children micronutrient status and anaemia has not been adequately studied. This study aimed to assess the contribution of integrated home gardening (IHG) intervention on anaemia, micronutrient intake and status in school children aged 5 to 12 years. Two sequential cross-sectional studies were conducted in school children aged 5-12 years from four study villages in Chamwino and Kilosa districts, Tanzania. The first survey was before and the second one a year after implementation of integrated home gardening intervention. Data were collected on dietary intake, haemoglobin and micronutrient status (e.g., vitamin A, iron, zinc). After the intervention, the overall prevalence of anaemia and vitamin A deficiency (VAD) decreased from 42.7 to 30.6%, and 24.5 to 0.4% respectively. Zinc remained a limiting nutrient as small proportion of children in Chamwino (12.5%) and Kilosa (3.3%) reached recommended intakes. The proportion of children consuming fruits and other vegetables significantly increased in both districts. The proportion of households reported to grow vegetables significantly increased from 76.6 to 82.1% , awareness on pocket gardening as a method of growing vegetables increased from 21.6 to 92.9% ; whereas the proportion of households practicing pocket gardening increased from 3 to 76.4%. Even though a statistically significant association between IHG intervention and micronutrient outcomes was not observed, a significant decrease in anaemia and VAD indicate an improvement in micronutrient status among school children. These results show that home gardening practices in combination with nutrition education can contribute to improvements in nutritional outcomes and should therefore be integrated into public health programmes.

Key words: School children, micronutrients, anaemia, home garden, intervention

4.1 Introduction

Undernutrition, which encompasses wasting, stunting, and micronutrient deficiencies, continues to be a prevailing condition in low-income and middle-income countries (Cabalda *et al*., 2011). Tanzania being one of the low-income country, anaemia and micronutrient deficiencies principally iron and vitamin A among school children are still a major public health problem (Munisi *et al*., 2016). A study among school children in Central Tanzania reported an overall 43% prevalence of anaemia (Mboera *et al*., 2015). This is in agreement with results of the scale-N project baseline study among school children in Dodoma and Morogoro regions in Tanzania, where an overall prevalence of anaemia (42.9%) and vitamin A deficiency (24.9%) was detected (Gowele *et al*., 2021). Anaemia and nutrient deficiencies during childhood can lead to retarded growth, poor cognitive development and reduced immune function that altogether may adversely affect academic performance through reduced learning capacity and poor school attendance (Osei *et al.*, 2010). Several factors contribute to under nutrition and anaemia among school children but there is still limited scaled-up experience about the appropriate interventions to address these problems countrywide. Deficiencies of micronutrients (MNs) such as iron, zinc and vitamin A can be driven by the presence of diseases, poor diet quality and limited food availability (Ferruzzi *et al.*, 2020). Inadequate dietary intake results in poor nutritional status of vulnerable populations (women and children); therefore, it is important to ensure both quantity and variety of foods available provide a range of essential nutrients.

In July 2016, the Scale-N project “Scaling-up Nutrition: Implementing potentials of nutrition-sensitive and diversified agriculture to increase food security” (2016–2018) conducted a baseline study to determine nutritional and micronutrient status and to identify nutritional gaps among the female small-scale farmers and their school children from study villages in Chamwino and Kilosa districts Tanzania. Results of the baseline survey

revealed several nutritional gaps including high prevalence of stunting, anaemia, micronutrient deficiencies (vitamin A, iron and zinc); low intake of fruits, vegetables and animal based foods; limited nutrition knowledge as well as inadequate skills related to food preparation, cooking, food allocation and undesirable food consumption practices (Bundala *et al.*, 2020; Gowele *et al.*, 2021). In response to the nutritional problems observed in the study population, an integrated home gardening intervention with nutrition education was implemented in the study areas.

Home gardening refers to the cultivation of a small portion of land which may be around the household or within walking distance from the family home that may serve as a strategy to enhance household food security (Galhena *et al.*, 2013). Home gardening is a sustainable strategy that can address multiple micronutrient deficiencies due to increased availability of fruits and vegetables (Cabalda *et al.*, 2011). Baliki *et al.* (2019) reported integrated home garden interventions which combine training in gardening practices with education about nutrition knowledge have shown to improve nutrition behaviour in low income countries. Promoting home gardening, along with awareness of human nutrition is commonly practiced in developing countries to encourage dietary diversity, allowing families to improve their food security, health and nutritional status (Thamilini *et al*., 2019). Studies indicate that home gardens benefit the nutrition of households in various ways; reducing micronutrients (MNs) deficiencies such as iron and vitamin A (Landon-Lane, 2011); provide access to green leafy vegetables that contain dietary sources of vitamins, minerals and other bioactive compounds (Egbi *et al.*, 2018). Moreover, provision of nutrition education helps to improve the knowledge of and practices related to nutrition of household members regarding the use of home-grown fruits and vegetables, thus improves the diets and nutritional status of household members (Baliki *et al.*, 2019).

This study used a pre and post comparison to evaluate the effect of an integrated home gardening intervention implemented by the Scaling-up Nutrition project (Scale-N) on child anthropometry, haemoglobin, anaemia, micronutrient intake and status particularly, vitamin A (retinol), carotenoids, vitamin E (α-tocopherol), iron and zinc. In addition, this study assessed the performance of home gardening practice indicators (e.g., pocket gardening awareness, proportion of households growing vegetables, households performing pocket gardening) among mothers or caregivers in Chamwino and Kilosa districts, Tanzania.

4.2 Methodology

This study was conducted in Mzula and Chinoje villages in Chamwino district and Mhenda and Tindiga villages in Kilosa district in Tanzania. In this follow-up study, two sequential cross sectional surveys were done. One was conducted during the baseline from July to August 2016 and another at endline from July to August 2018. The intervention phase took place from July 2017 to May 2018. Details of the study areas, sample and field procedure have been described in our previous publication (Stuetz *et al*., 2019). Briefly, a total of 666 households having a mother or a caregiver and a schoolchild aged between 5–10 years who participated in the Scale-N project baseline study were eligible for the intervention.

The study was ethically approved by the National Institute for Medical Research (NIMR) Tanzania (Certificate number, NIMR/HQ/R.8a/Vol.IX/2226), and the Ethics Committee Landesärztekammer Baden-Württemberg, Stuttgart, Germany (F-2016-049). Permission was also obtained from the regional and district Departments of Health. The purpose of the study was explained to the mother or caregiver of each child, and written informed consent was obtained before the interview.

4.3 Description of the Intervention

The integrated home gardening intervention included two main components: (a) nutrition education and (b) home gardening to improve diet and micronutrient status of the study population. A community-based participatory approach was applied in implementing the integrated home gardening intervention in the study areas. The initial step involved dissemination of baseline research results to men and women from participating households. Thereafter, mapping of factors that affect the vegetable intake and eventually micronutrient status of the study population was done in a participatory manner. This step was followed by involvement of both men and women in the plan of the integrated home gardening intervention as a measure to improve vegetable intake and eventually micronutrient status. The nutrition education component whose implementation process and results are described in detail by Bundala *et al.* (2020) consisted of 5 main topics: (a) importance of good nutrition to the individuals, households and community, (b) malnutrition, (c) food preparations and cooking, (d) food consumption and (e) household food production.

The home garden component involved five key activities: 1) introduction of the home garden intervention to the participating households and village officials, 2) participatory selection of demonstration sites, 3) preparation of training materials and farm inputs (fliers, polyethylene sacks, seeds), 4) training on compost manure preparation and starting of vegetable nurseries and 5) training on how to set up a garden. Men and women from the participating households were trained on how to set up three kinds of home gardening systems; these are, sack or pocket, raised bed and brick gardens. However, this study focused mainly on the implementation of pocket gardening because the study areas had a major challenge of water scarcity which impaired households to grow nutritious vegetables. . Pocket or sack gardening employ minimal use of water thus has the potential

to overcome water scarcity. In addition, pocket garden is placed vertically which is suitable for gardeners with limited horizontal space thus optimizes the growing space where vegetables grow out of holes on the sides of the pockets and on the top. Other potentials of pocket gardens include the use of easily accessible materials such as sand, pebbles, manure, and soil that can be found within the localities.

Training and demonstrations on the use of pocket gardens in growing vegetables was done using a participatory approach where household members were grouped by hamlets with maximum 30 households per session. A total of 559 households were trained on pocket gardening and ultimately 443 households performed pocket gardening. Limited access to water was the most important challenge for the drop in adopters during the intervention phase. During the training sessions each participating household received polyethylene bags and vegetable seeds (spinach and Chinese cabbage). After receipt of garden inputs and training, participants started their own pocket gardens by planting vegetable seeds in the holes on the sides of the pockets and on the top. The pockets were placed within the homestead of the participating households. Participants were also encouraged to plant other vegetables such as sweet potato leaves '*Matembele'* at the bottom surrounding the earth-filled pocket bag to utilize the water that trickles down hence increasing vegetable varieties.

The entire study included three phases, (1) a baseline study to assess micronutrient status and to identify nutritional gaps, (2) an intervention phase (2017) for nutrition education and vegetable gardening, and (3) a follow-up to study the influence brought by the intervention. Therefore, pre and post analyses were done to examine the micronutrient and nutritional status before and after the intervention. The flow diagram of participants in the three phases of this study is presented in Figure 4.1.

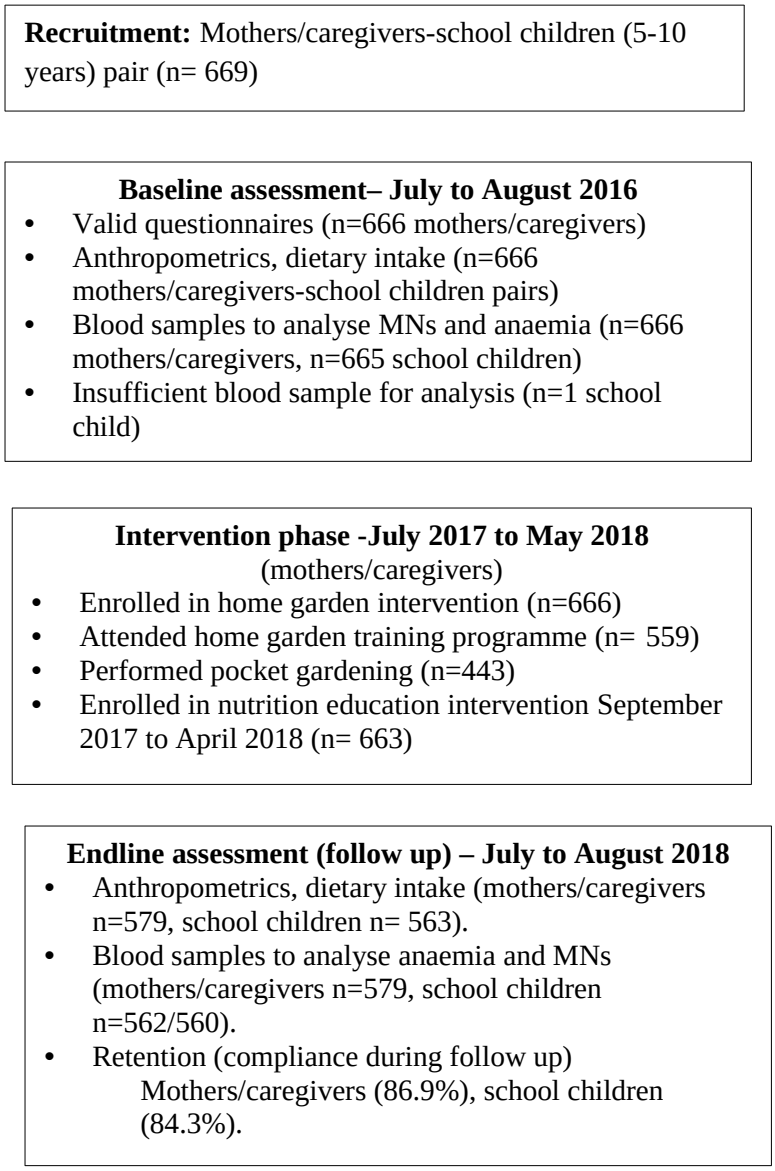

Figure 4.1: Participants flow diagram in a home gardening intervention integrated with nutrition education

4.3.1 Socio-demographic information

Socio-demographic information and dietary intake patterns of the study population were collected using the pre-tested structured Scale-N project questionnaire. The questionnaire was interview based and prepared in both English and Swahili version. The Swahili version was the one posed during the interview. All field assistants including those who took the anthropometric measurements received intensive training prior the survey following the guidelines endorsed by FANTA III, Training Package for Facility-Based

Service Providers (FANTA, 2015). The questionnaire included questions about home gardening practice indicators such as mothers or caregivers reported to grow vegetables; awareness about pocket gardens and practiced pocket gardening; and information on the child's age, sex, and reported malaria (in the last 3 months) and diarrhoea (in the last 4 weeks).

4.3.2 Anthropometric measurements

Weight was measured using an electronic SECA weighing scale (seca GmbH and Co. KG, Hamburg, Germany) to the nearest 0.1 kg in light clothing. Height was measured using a stadiometer (Model S0114540, UNICEF, New York City, USA). A child was measured while standing on a stadiometer without shoes, and the measurement was recorded to the nearest 0.1 cm. Anthropometric indices, height-for-age (HAZ), and body mass index-for-age, (BAZ) Z-scores were computed using WHO AnthroPlus (v1.0.4) software; nutritional status indices for overweight (> +1SD BMI-for-age Z score), obesity (> + 2SD BMI-for-age Z score), thinness/ wasting (< −2SD of BMI-for-age Z score), and stunting (< −2SD of height-for-age (HAZ) Z score) were defined according to the WHO reference growth charts for children aged 5 to 19 years (WHO, 2007).

4.4 Dietary Assessment

The macro and micronutrient intake of the target child and the dietary diversity of the household were assessed using data from a single 24-h dietary recall method. The 24-hour macro- and micronutrient intake (vitamins and minerals) of all reported foods per amount (grams, litres) for each child were calculated using the 'NutriSurvey' software package (Erhardt, 2007). This software contains all reported foods and recipes listed in the Tanzanian food composition tables (Lukmanji *et al.*, 2008). In addition, micronutrient contents of indigenous leafy vegetables consumed in the study area (Gowele *et al.*, 2019)

were also entered in the NutriSurvey software. The adequacy of micronutrient intake was analysed using the recommended nutrient intakes (RNIs) by WHO (WHO, 2004) whereas for macronutrients, the percentage of total energy intake from proteins, carbohydrates, and total fat was evaluated using the acceptable macronutrient distribution ranges (AMDR) for children aged 4 to 18 years: 10–30% for protein, 25–35% for fat, and 45–65% for carbohydrate (IoM, 2005). Furthermore, to capture the dietary pattern of the participating households, a set of 12 food groups was used to compute household dietary diversity score (HDDS) by following the standard tool suggested by the Food and Agricultural Organization to measure dietary diversity (Kennedy *et al.*, 2013). The 12 food groups considered in this study were, cereals; vegetables (dark green leafy vegetables and other vegetables); fruits (vitamin A rich fruits and other fruits); white tubers and roots; any meat and organ meat; eggs; any fish; legumes, nuts and seeds; milk and milk products; oils and fats; sugar or honey; spices, condiments and beverages.

4.4.1 Biochemical assessments

3-5 ml venous blood samples of mothers or caregivers and school children were drawn for analysis of micronutrient status and infection markers. Haemoglobin (Hb) concentrations were measured on site using Hemocue method. Anaemia was defined as Hb <115 g/L for children aged 5-11 years and < 120 g/L for children 12–14 years; Hb concentrations in one village (Mzula) were adjusted for altitude by subtracting 2 g/L from respective haemoglobin measurements as per WHO recommendation (WHO, 2017). The concentrations of retinol, vitamin E (α-tocopherol) and carotenoids in serum were determined in Hohenheim, Germany using the high-performance liquid chromatography (HPLC); while serum aliquots were analysed (at the VitMin Lab, Dr. JG Erhardt, Willstaett, Germany) on iron status markers (ferritin; sTfR, soluble transferrin receptor), infection or inflammation markers (CRP, C-reactive protein; AGP, α-1 glycoprotein) by a

sandwich enzyme-linked immune-sorbent assay technique, and serum zinc by a spectrophotometric method as previously described in detail (Erhardt *et al*., 2004; Gowele *et al*., 2021).

Serum ferritin was adjusted for respective correction factors for children and the 3 different inflammation stages: factor 0.64 for incubation (CRP > 5 mg/L and AGP ≤ 1 g/L), 0.39 for early convalescence (CRP > 5 mg/L and AGP > 1 g/L), and 0.65 (CRP ≤ 5 mg/L and AGP > 1 g/L) for late convalescence (Thurnham *et al.*, 2010). Iron deficiency (ID) was defined as serum ferritin <15µg/L or sTfR >8.5 mg/L for children > 5 years (WHO, 2001) and total body iron stores (IST) were calculated by an equation using ferritin and sTfR (Cook *et al.*, 2003). Elevated acute phase proteins CRP > 5 mg/L and AGP >1 g/L were used as indicators for an acute phase response by infection or inflammation (Thurnham *et al.*, 2010). Retinol <0.7 µmol/L was considered indicative of vitamin A deficiency (VAD) (WHO, 1996) while serum zinc <0.65 mg/L was used to indicate low/deficient zinc status for all age and sex groups combined (Hotz *et al.*, 2003). Vitamin E deficiency was considered when α-tocopherol was less than 11.6 µmol/L (MC Lobo *et al.*, 2019). Total provitamin A carotenoid (sum of α-carotene, β-carotene, and β-cryptoxanthin) was considered sufficient within the reference range for serum carotene (0.9–3.7 µmol/L) used to assess habitual intake of fruits and vegetables (Mondloch *et al.*, 2015).

4.4.2 Data analysis

Socio-demographic characteristics; anthropometrics (HAZ, BAZ); home gardening practice indicators; blood biomarkers serum ferritin (SF) and soluble transferrin receptor (sTfR) as indicators for iron status; C-reactive protein (CRP) and α-1 glycoprotein (AGP) as indicators for infection markers; serum micronutrients (retinol, carotenoids, tocopherols, zinc) and data on dietary intake of macro and micronutrients of the study participants are

described using medians with interquartile range (IQR) and percentage (number), as appropriate. Comparisons between the baseline and post intervention phase were done using the chi-squared and McNemar test for categorical data and the prevalence of deficiencies whereas for continuous variables the Wilcoxon Signed Ranks test was used. Logistic regression analysis was carried out to investigate the factors that predict anaemia and micronutrient deficiencies. In separate models anaemia, iron deficiency and zinc deficiency were included as dependent variables while acute phase proteins (AGP >1 g/L, CRP >5 mg/L), serum micronutrients, dietary micronutrient intake and home garden practice indicators were included as independent variables. A forward stepwise approach was used and only variables with P values < 0.05 were retained in the final models. The strength of association between potential predictive factors and nutrition status were measured by the Odds Ratio (OR) and their 95% confidence interval (CI). Appropriate fit of logistic regression models was confirmed using the Hosmer–Lemeshow goodness-of-fit test. All data were entered and analysed using SPSS software (SPSS Inc., Chicago, IL, USA; Version 20.0.0) and p values <0.05 were considered as statistically significant.

4.5 Results

In July-August 2016 a total of 666 mother/caregiver-child pairs participated in the baseline survey, and, in July-August 2018, 579 out of 666 (86.9%) mothers or caregivers and 563 out of 666 (84.5%) school children participated in the endline survey. The overall median age of school children at the endline survey was 9.2 years.

4.6 Anthropometric Data

In both districts, the proportion of stunted children did not differ significantly (Table 4.1). In Chamwino no significant changes were observed in the proportion of wasted and overweight children whereas in Kilosa, the proportion of overweight children was lower at endline and the difference was statistically significant (Table 4.1).

Table 4.1: School children's anthropometric status at baseline and follow-up surveys

Demographic and nutrition indicators		Chamwino		Kilosa		Total	
		N=289		N=273		N=562	
		Baseline	Endline	Baseline	Endline	Baseline	Endline
Age (years)	Median	7.3	9.3	7.2	9.2	7.3	9.2
	IQR	6.5 - 8.1	8.5 -10.1**	6.4 - 8.1	8.4 - 10.1 **	6.5 - 8.1	8.4 - 10.1 **
Stunting	%	22.9	24.9	29.6	30.7	26.1	27.7
Wasting	%	1.0	2.8	0	1.1	0.5	2.0*
Overweight	%	2.4	3.1	8.0	2.2	5.2	2.7*

P values: Wilcoxon Signed Ranks Test (median) or McNemar test (prevalence) as appropriate. Significant differences are presented by p values *p < 0.05 and ** p < 0.001. IQR = Interquartile range; Stunting = HAZ< -2 SD; Wasting = BAZ< −2SD; Overweight = 1 SD < BAZ ≤ 2 SD (WHO, 2007).

4.7 Biochemical Markers

Results from the endline survey revealed significant improvements in haemoglobin and zinc concentration and eventually a decrease in anaemia prevalence and zinc deficiency (<0.65 mg/L) in children from Kilosa. Children in Chamwino had a significant decrease in zinc concentration; this was also reflected in a significant increase in zinc deficiency. Markers of inflammation (AGP > 1 g/L or CRP > 5 mg/L), serum ferritin and total body iron stores did not vary between surveys. Nevertheless, the concentration of soluble transferrin receptor (sTfR) was significantly higher in Chamwino. Serum retinol and α-tocopherol concentrations significantly improved in both districts. Eventually, the prevalence of vitamin A deficiency (retinol, <0.7 µmol/L) and vitamin E deficiency (α-tocopherol < 11.6 µmol/L) decreased significantly (Table 4.2).

Table 4.2: Biochemical markers in school children between surveys and across districts

Biochemical parameters		Chamwino N=289		Kilosa N=273		Total N=562	
		Baseline	**Endline**	**Baseline**	**Endline**	**Baseline**	**Endline**
Haemoglobin, adj. (g/L)	Median	122	120	112	120	117	120
	IQR	115 - 128	114 - 127	105 - 118	111 - 127 **	109 - 124 **	112- 127
Anaemia	%	24.6	28.4	61.9	33.0 **	42.7	30.6 **
	N	**288**		**272**		**560**	
CRP >5 mg/L	%	7.3	5.9	16.2	16.9	11.6	11.2
AGP >1g/L	%	11.8	17	33.1	26.8	22.1	21.8
Ferritin, adj. (µg/L)	Median	34.5	37.7	33.8	30.7	34.0	34.8
	IQR	25.4 - 53.3	24.8 - 57.2	23.4 - 51.3	20.2 - 52.4	24.5 - 52.2	22.2 - 54.8
sTfR (mg/L)	Median	6.7	7.1	7.9	7.8	7.2	7.4
	IQR	5.8 - 7.9	6.1- 8.2 *	6.6 - 10.3	6.6 - 9.5 *	6.2 - 8.7	6.3 - 8.9
ID	%	20.1	25.3	41.5	46.3	30.5	33
IST (mg/kg BW)	Median	4.5	4.5	3.7	3.6	4.1	4.0
	IQR	3.0 - 6.1	2.8 - 6.2	2.1- 5.1	1.4 - 5.4	2.5 - 5.6	2.0 - 5.9
Zinc (mg/L)	Median	0.71	0.68	0.72	0.75	0.72	0.72
	IQR	0.64 - 0.8	0.6 - 0.78 *	0.65 - 0.80	0.67- 0.84 *	0.63 - 0.80	0.62- 0.81
Zinc <0.65 mg/L	%	28.1	39.2 *	24.3	20.6	26.2	30.2
Retinol (µmol/L) [1]	Median	0.82	1.28	0.87	1.44	0.85	1.36
	IQR	0.68 - 0.98	1.13 - 1.45 **	0.73 - 0.97	1.28 - 1.65**	0.7- 0.98	1.19 - 1.56 **
VAD	%	27.8	0.7**	21	0	24.5	0.4**
α-tocopherol, µmol/L	Median	14.2	16.9	16.9	20.6	15.5	18.5
	IQR	12.3 - 16.1	14.6 - 20.0 **	14.7- 19.7	17.7- 23.6 **	13.3 - 18.0	15.9- 21.7 **
α-tocopherol, <11.6 µmol/L	%	17	3.1**	5.9	0.4**	11.6	1.8**
γ-tocopherol, µmol/L	Median	1.1	1.2	0.4	0.5	0.7	0.8
	IQR	0.8 - 1.7	0.9 - 1.7	0.3 - 0.6	0.4 - 0.7 *	0.4 - 1.2	0.5 - 1.2*

P values: Wilcoxon Signed Ranks Test (median) or McNemar test (prevalence) as appropriate. Significant differences are presented by p values *p < 0.05, and ** p < 0.001. IQR = Interquartile range; Anaemia, haemoglobin <115 and 120 g/L; VAD, vitamin A deficiency, retinol, <0.7 µmol/L; sTfR, soluble transferrin receptor.ID, iron deficiency (=1, yes), if serum ferritin (adjusted) < 15 µg/L or sTfR >8.5 mg/L; IST, total body iron stores. CRP, C-reactive protein; AGP, α-1 glycoprotein.

4.8 Serum Carotenoids

During the endline survey in 2018, significant improvements in serum α-carotene and β-carotene concentration in children from both districts were observed. Lutein-zeaxanthin concentration was significantly higher in Children from Kilosa. Moreover, the proportion of children with total provitamin A concentration meeting the common reference range for serum carotene (0.9–3.7 μmol/L) increased significantly in Kilosa. Nevertheless, a significant decrease in β- cryptoxanthin and lycopene concentrations were observed in both districts (Table 4.3).

Table 4.3: Serum carotenoids of school children before and after the implementation of integrated home gardening intervention

Carotenoids concentration		Chamwino N=288		Kilosa N=272		Total N=560	
		Baseline	Endline	Baseline	Endline	Baseline	Endline
α-carotene, µmol/L	Median	0.04	0.05	0.29	0.40	0.11	0.17
	IQR	0.03 - 0.06	0.04 - 0.07 **	0.19 - 0.46	0.26 - 0.64 **	0.04 - 0.29	0.05 - 0.40 **
β-carotene, µmol/L	Median	0.53	0.62	0.46	0.59	0.49	0.61
	IQR	0.38 - 0.72	0.46 - 0.79 **	0.32- 0.66	0.42- 088 **	0.34 - 0.69	0.44 - 0.84 **
β- cryptoxanthin, µmol/L	Median	0.17	0.07	0.18	0.15	0.17	0.09
	IQR	0.08 - 0.37	0.05 - 0.10 **	0.08 - 0.33	0.08 - 0.31 *	0.08 - 0.36	0.06 - 0.17 **
Lutein/zeaxanthin, µmol/L	Median	1.18	1.21	0.68	0.77	0.93	1.0
	IQR	0.95 - 1.50	0.93 - 1.61	0.51 - 0.89	0.50 - 1.07 **	0.64 - 1.26	0.70 - 1.38 **
Lycopene, µmol/L	Median	0.23	0.10	0.53	0.49	0.33	0.22
	IQR	0.16 - 0.35	0.06 - 0.17 **	0.34 - 0.77	0.28 - 0.70 *	0.19 - 0.57	0.10 - 0.49 **
Provitamin A carotenoids, µmol/L	Median	0.82	0.76	1.04	1.24	0.90	0.93
	IQR	0.58 - 1.11	0.58 - 1.00	0.67-1.45	0.88 - 1.87 *	0.62 - 1.24	0.66 - 1.38 *
Provitamin A carotenoids, 0.9-3.7µmol/L	%	41.3	35.4	58.5	71**	49.6	52.7

P values: Wilcoxon Signed Ranks Test (median) or McNemar test (prevalence) as appropriate. Significant differences are presented by p values *p < 0.05, and ** p < 0.001. IQR = Interquartile range; Provitamin A carotenoids (total α-Carotene, µmol/L, β-Carotene, µmol/L and β-Cryptoxanthin, µmol/L).

4.9 Macro- and Micronutrient Intake

The comparison of children's macro- and micronutrient intakes between surveys are presented in Table 4.4 and Table 4.5. At endline, a small proportion of children in Chamwino (12.2%) and Kilosa (7.7%) met the recommended energy intake across districts. In Kilosa, protein and fat consumption was below the lower limit, while carbohydrate intake exceeded the upper limit of the acceptable macronutrient distribution ranges of energy intake. Notably, significant improvements in median intakes of energy, protein, fat, carbohydrate, vitamin E, vitamin B_2, B_6, folic acid, ascorbic acid and zinc were observed in Chamwino. In contrast, vitamin E and zinc remained the most limiting nutrients in Kilosa as the proportion of children meeting recommended intakes decreased to below 4% at endline. Furthermore, the proportion of children meeting RNI for iron significantly decreased in both districts.

Table 4. 4: School children's macronutrient intakes between surveys

N		Chamwino 288		Kilosa 271		RNI/AMDR
Nutrient		**Baseline**	**Endline**	**Baseline**	**Endline**	
Energy, Kcal	Median	610	1063	1209	1195	1250-2350
	IQR	424 - 870	710 - 1498 **	941- 1543	965 - 1481	
Energy ≥ RNI	%	2.8	12.2 **	16.2	7.7 *	
Protein (g)	Median	14.2	23.7	27.9	28.2	19-34
	IQR	9.5 - 21.1	15.6 - 43.7 **	19.9 - 44.7	20.6 - 38.7	
Protein ≥ RNI	%	23.6	40.3 **	66.8	48 **	
% EN by Protein	Median	12	11	10	9	10–30%
	IQR	10 - 14	9 - 14 *	7 - 13	8 - 11	
Fat (g)	Median	10.5	27.2	25.3	24.6	
	IQR	5.7 - 18.2	16.2 - 58.1 **	17.2 - 39.2	17.8 - 36.3	
% EN by fat	Median	18	30	19	18	25–35%
	IQR	11 - 26	20 - 42 **	14 - 25	14 - 24	
Carbohydrates (g)	Median	83.3	131.8	206.8	213.9	130
	IQR	53.4 - 127.9	88.6 - 188.4 **	165.2 - 260.9	174.4 - 263.8	
% EN by CHO	Median	71	59	70	73	45–65%
	IQR	61 - 76	45 - 70 **	65 - 75	67 - 76 *	

P values: Wilcoxon Signed Ranks Test (median) or McNemar test (prevalence) as appropriate. IQR = Interquartile range; significant differences are presented by p values * $p < 0.05$ and ** $p < 0.001$. RNI /day, recommended macronutrient intake adjusted for sex and age (4-6, 7-9, 10-12 years) and acceptable macronutrient distribution ranges (AMDR) following the IoM recommendations (IoM, 2005).

Table 4.5: School children's micronutrient intakes between surveys

N		Chamwino 288		Kilosa 271		RNI/AMDR
Nutrient		**Baseline**	**Endline**	**Baseline**	**Endline**	
Vitamin A (μg)	Median	254	302	108	131	400 - 600
	IQR	150 - 417	154 - 502	26 - 312	47 - 291	
RE ≥ RNI	%	21.5	21.9	16.6	10.3 *	
Vitamin E (mg)	Median	0.46	2.39	0.84	0.83	5 - 7.5
	IQR	0.02 - .82	0.91 - 9.58 **	0.5 - 1.54	0.5 - 1.66	
α-TE ≥ RNI	%	5.2	27.1 **	5.2	3.7	
Vitamin B2 (mg)	Median	0.56	0.67	0.44	0.44	0.6 - 1
	IQR	0.38 - 071	0.43 - 1.04 **	0.25 - 0.66	0.28 - 0.65	
B2 ≥ RNI	%	25	29.5	21.8	10.3 **	
Vitamin B6 (mg)	Median	0.66	0.93	0.87	0.95	0.6 - 1.2
	IQR	0.46 - 0.89	0.62 - 1.29 **	0.63 - 1.12	0.71 - 1.26 *	
B6 ≥ RNI	%	35.1	40.6	54.2	39.9 *	
Folic acid (μg)	Median	185	203	206	206	200 - 400
	IQR	118 - 252	131 - 332 *	138 - 338	134 - 319	
FA ≥ RNI	%	24.7	24.3	39.5	23.6 **	
Ascorbic acid (mg)	Median	2.8	34.8	19.8	24.1	30 - 40
	IQR	0.7 - 8.6	12.5 - 153 *	7.1 - 41.2	12.2 - 57.1 *	
AA ≥ RNI	%	12.8	47.9 **	32.8	35.8	
Iron (mg)	Median	16.6	16.5	8.1	7.7	6 - 14
	IQR	11.7 - 23.2	11.2 - 23.8	5.8 - 11.3	5.9 - 10.3	
Iron ≥ RNI	%	91.7	77.8 **	54.2	25.8 **	
Zinc (mg)	Median	4.48	6.37	5.19	5.50	10.3 -15.5
	IQR	3.28 - 6.20	4.30 - 9.53 **	3.74 - 6.92	4.38 - 7.37 *	
Zinc ≥ RNI	%	4.2	12.5	4.4	3.3	
Calcium (mg)	Median	499	372	161	199	600 -1300
	IQR	273 - 764	230 - 605 *	83 - 371	123 - 445	
Calcium ≥ RNI	%	32.6	13.2 **	16.6	13.3	

P values: Wilcoxon Signed Ranks Test (median) or McNemar test (prevalence) as appropriate. IQR = Interquartile range; significant differences are presented by p values * $p < 0.05$ and ** $p < 0.001$. RNI /day, recommended daily nutrient intake regarding micronutrient adjusted for sex and age (4-6, 7-9,10-12 years) following the WHO recommendations (WHO and FAO, 2004); for zinc the low bioavailability and for iron the 10% bioavailability was applied.

4.10 Food Consumption and Dietary Diversity

The frequency and quantities of consumed food items in the two districts are summarized in Table 4.6. At endline, the proportion of children reported to consume vegetables other than DGLV and fruits, and the median values for household dietary diversity score (HDDS) significantly increased in both districts. In Chamwino, children consumed most frequently dishes that comprised bulrush millet, dark green leafy vegetables (DGLV) and legumes. In Kilosa, diet consisted of maize, rice or roots in combination with higher quantities of other vegetables and/or legumes compared to Chamwino. Meat and fish were more frequently consumed in Kilosa while milk and eggs were consumed more in Chamwino, but generally, in both districts, their consumption was rare.

Table 4.6: Food items consumed by school children between surveys

		Chamwino		Kilosa	
N		288		272	
Food		Baseline	Endline	Baseline	Endline
[1] Millet (g)	Median	375	417	250	-
	IQR	250 - 500	250 - 500	213 - 375	
Millet	%	91	84 *	4.4	0
[2] Maize (g)	Median	400	500	250	250
	IQR	100 - 500	63 - 625	125 - 430	125 - 450
Maize	%	12	12	87	88
[3] Rice (g)	Median	213	250	250	250
	IQR	94 - 225	188 - 188	250 - 300	250 - 375
Rice	%	4	7	61	65
[4] Roots (g)	Median	83	100	150	150
	IQR	33 - 106	50 - 125	100 - 200	100 - 200
Roots	%	1.7	2.4	24	30
[5] DGLV (g)	Median	125	110	100	92
	IQR	90 - 202	63 - 180 *	63 - 132	63 - 125
DGLV, % (n) [2]	%	91	93	34	43
[6] Other vegetables (g)	Median	63	30	100	69
	IQR	38 - 138	30 - 83	39 - 100	50 - 125
Other vegetables	%	8	47 **	28	36 *
[7] Fruits (g)	Median	134	115	152	100
	IQR	66 - 200	95 - 169	91 - 200	70 - 200
Fruits	%	10	41 **	17	29 **
[8] Legumes (g)	Median	20	78	128	125
	IQR	10 - 103	15 - 250 **	100 - 221	63 - 200
Legumes	%	55	89 **	76	73
[9] Meat (g)	Median	50	75	55	75
	IQR	31 -75	50 - 100	43 - 100	55 - 113
Meat	%	5	11 **	15	17
[10] Fish (g)	Median	100	81	125	83
	IQR	63 - 110	63 - 100	100 - 150	63 - 130
Fish	%	2	6	24	30
[11] Milk and eggs	%	3.8	4.5	0.4	1.8
HDDS	Median	4.0	5.0	6.0	7.0
	IQR	3.0 - 5.0	4.0 - 6.0 **	5.0 - 7.0	6.0 - 7.0 **

P values: Wilcoxon Signed Ranks Test (median) or McNemar test (prevalence) as appropriate. IQR = Interquartile range; significant differences are presented by p values *p < 0.05 and ** p < 0.001.[1] Millet includes pearl and finger millet dishes; [2] maize includes stiff porridge '*Ugali*' and soft porridge dishes; [3] rice cooked with coconut or oil and onions; [4] roots include cassava, potato and yams; [5] Dark green leafy vegetables (DGLV) include '*Mlenda*' or '*Ilende*', amaranth, cow pea, sweet potato and pumpkin leaves or spinach; [6] Other vegetables: okra, pumpkin, tomato, African eggplant, Chinese or white cabbage; [7] Fruits include banana, baobab, guava, mango, papaya, water melon; [8] Legumes include beans, peas, bambara nut and ground nut; [9] Meat include beef, goat, chicken and pork; [10] Fish include fish relish and dried sardines; [11] Milk include milk products.

4.11 Risk Factors of Anaemia, ID and ZnD

Multiple logistic regression models to identify factors being significantly associated with anaemia, ID and ZnD are summarized in Table 4.7. Elevated α-1 glycoprotein (AGP> 1 g/L) was highly associated with anaemia and ID in Chamwino. Moreover, sufficient dietary intakes of protein and folate reduced the risk, while zinc deficiency increased the risk of anaemia in Kilosa. Livestock ownership and calcium intake reduced the risk for ZnD in Chamwino whereas in Kilosa, ZnD was predicted by infection (AGP> 1 g/L). Notable, the inverse association of serum α- tocopherol with ZnD and household dietary diversity score (HDDS) with ID in Kilosa. Variables on home gardening practice were also included in the models but the association could not be demonstrated as statistically significant.

Table 4.7: Determinants of anaemia, iron deficiency (ID) and zinc deficiency (ZnD) in school children at endline

District	Micronutrient status marker	Beta	95% CI
Chamwino N=288	**Anaemia, adj (28.4%)**		
	High AGP, =1 (yes)	2.23	1.18 to 4.21
	ID, adj (25.3%)		
	High AGP, =1 (yes)	3.01	1.57 to 5.78
	ZnD (39.2%)		
	Own livestock, =1 (yes)	0.56	0.34 to 0.92
	Ca intake ≥ RNI, =1 (yes)	0.38	0.16 to 0.89
Kilosa N= 272	**Anaemia, adj (33.0%)**		
	ZnD, =1 (yes)	2.33	1.13 to 4.82
	Protein intake ≥ RNI, =1 (yes)	0.42	0.20 to 0.86
	Folate intake ≥ RNI, =1 (yes)	0.24	0.07 to 0.88
	ID, adj (46.3%)		
	HDDS	0.78	0.62 to 1.0
	ZnD (20.6%)		
	High AGP, =1 (yes)	4.30	2.15 to 8.61 *
	α-tocopherol µmol/l	0.89	0.81 to 0.97

Multiple logistic regression models with a forward approach; values are Exponential Beta = odds ratio (95% CI); all factors at $p<0.05$, except * $p<0.00$. Variables in the initial models included child age, reported malaria (in the last 3 months) or diarrhoea (in the last 4 weeks), home garden practice indicators (household grow vegetables and practice pocket gardening, no=0 versus yes=1), CRP > 5 mg/L (no=0 versus. yes=1); MN deficiencies (ID =1, iron deficiency (no=0 versus yes=1); VAD =1, vitamin A deficiency (no=0 versus yes=1); serum micronutrients (e.g., zinc), and sufficient dietary intakes of assessed MNs (e.g., iron or vitamins ≥ RNI).

4.12 Home Gardening Practice Indicators

After the intervention, the total proportion of mothers or caregivers reported growing vegetables significantly increased (Chi-square test, $p < 0.017$). Moreover, the proportion of mothers or caregivers who were aware and practiced pocket gardening as a method of growing vegetables significantly increased (Chi-square test, $p < 0.001$) in both districts. More mothers or caregivers reported raising livestock in Chamwino than in Kilosa, but overall, the differences were statistically non-significant (Figure 4.2).

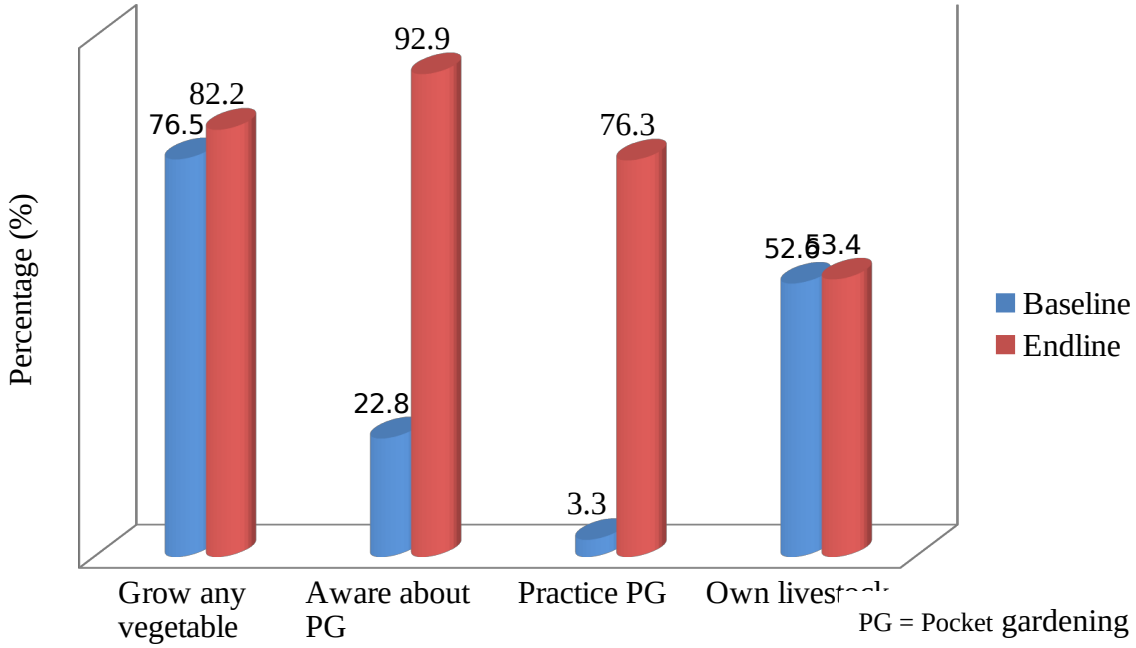

Figure 4.2: Home gardening practice indicators by households prior and after the integrated home gardening intervention.

4.13 Discussion

This study aimed to assess the contribution of integrated home gardening (IHG) intervention on anaemia, micronutrient intake and status in school children enrolled along with their mothers or caregivers in the Scale-N project in 2016. After one year implementation of IHG intervention, no significant changes were observed in relation to children anthropometry as the prevalence of stunting remained unchanged whereas wasting and overweight were infrequent.

This study further highlights a significant decrease in overall prevalence of anaemia and VAD. This is simultaneously reflected by the significant improvements in child haemoglobin, serum retinol and provitamin A carotenoids particularly α-carotene and β-carotene concentrations. The higher serum α-carotene and β-carotene in children from both districts as well as higher serum lutein-zeaxanthin concentration in children from Kilosa is most likely due to the frequent consumption of DGLV, other vegetables and fruits as reported in the endline survey. These findings are supported with results from a controlled intervention study investigating the associations between daily fruit and vegetable intake and circulating carotenoid concentrations in men and women where authors concluded that serum α-carotene, β-carotene and lutein-zeaxanthin are reliable biomarkers of mixed fruit and vegetable consumption (Couillard *et al*., 2016).

Despite the significant decrease in anaemia its prevalence (30.6%) is still a public health concern among school children in the study area. In Chamwino, children with elevated AGP were at higher risk of anaemia whereas in Kilosa children with ZnD were at more risk of being anaemic. Moreover, sufficient intake of protein and folate was associated with the lower risk of anaemia in Kilosa. The observed risk factors of anaemia suggest that improving dietary sources protein (foods of animal origin, legumes) and folate (organ meat, green vegetables, whole grains, legumes) in children's diet could help to control the problem of anaemia. Furthermore, the dietary measures in addressing the problem of anaemia should be in parallel with the control of infections.

The prevalence of iron and zinc deficiency in school children increased significantly despite the implementation of IHG intervention. The prevalence of ID in Kilosa was significantly higher than in Chamwino (46.5% vs. 25.3%) as reflected by the significant decrease in the proportion of children meeting RNI for iron in both districts. Zinc

deficiency was significantly higher in Chamwino than in Kilosa (39.2 % vs. 20.6%). Simultaneously, lower median serum zinc concentration was observed in Chamwino than in Kilosa (0.68 mg/L vs. 0.75 mg/L) and only 12.5% of children in Chamwino and 3.3% in Kilosa were able to meet RNI for zinc. The high prevalence of iron and zinc deficiency in both districts, can further be explained by the frequent consumption of plant-based diets consist mainly of cereals, vegetables and legumes with limited consumption of animal-based foods (red meat, poultry, fish) as dietary sources of iron and zinc. At endline, the median intake in the amount of DGLV, other vegetables and legumes were far below recommendation of 400g per day while for meat and fish were below 100-150g per day (WHO, 2003) to improve the intake of iron and zinc among school children in the study area.

In Kilosa, children with elevated AGP were at higher risk of ID whereas an increase in HDDS was associated with lower risk of ID. The observed risk factors for ID suggests that improving dietary diversity through consumption of iron rich foods and control of coexisting infections such as malaria and diarrhoea, as reported in this population previously (Gowele *et al*., 2021) are important factors to consider in improving iron status of the study population. In Chamwino, livestock ownership (chicken/ducks or cattle/goats/pigs) and sufficient calcium intake (intake ≥ RNI) was associated with lower risk of ZnD. This suggests similarities in dietary sources of zinc and calcium as whole grains/cereals, vegetables, meat and dairy products are their main dietary sources (Roohani *et al*., 2013; Melse-Boonstra, 2020).

Nevertheless, results at endline show infrequent consumption of milk and eggs which are good sources of animal protein and calcium necessary during the growth phase of children. Notable, zinc absorption in humans is substantially higher in the presence of protein from

animal sources than plant-based protein thus addition of animal protein to vegetable-based food significantly improves bioavailability of zinc (Maares and Haase, 2020).

Furthermore, zinc deficiency was inversely associated with serum α-tocopherol and was predicted by infection (AGP >1 g/L) in Kilosa. This association is literally linked to the role played by zinc and α-tocopherol in anti-inflammatory effects and as potent antioxidants (Shamim *et al*., 2013; Chin and Ima-Nirwana, 2018).

The IHG intervention significantly increased the proportion of households reported to grow vegetables. Moreover, awareness and adoption of pocket gardening also increased significantly. The findings observed after one year implementation of IHG intervention suggest the possibility that more positive results on children's nutrition and health outcomes would have been achieved with a longer exposure to the intervention.

4.14 Conclusions and Recommendations

Findings of the present study suggest an important contribution of IHG intervention on the improvement of diets and micronutrient status among school children living in rural farming households. Significant decrease in the prevalence of anaemia and vitamin A deficiency as reflected by the significant increase in retinol and haemoglobin concentration was observed at the time of the follow-up survey. Moreover, the higher overall serum α-tocopherol, α-carotene, β -carotene and lutein-zeaxanthin concentration was simultaneously reflected with significantly increased proportion of children reported to consume legumes, fruits and vegetables other than DGLV. Elevated AGP concentration remained the strongest predictor of anaemia, ID and ZnD thus concurrent programs that reduce infectious diseases and improve hygiene are required.

Author Contributions:

V.F.G. and W.S. were responsible for the study design, carried out the field survey, performed laboratory-, data and statistical analysis. V.F.G. wrote the manuscript. W.S., J.K., T.J., CR and S.S. provided technical assistance for the overall study design and provided critical feedback on the manuscript.

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CHAPTER FIVE

5.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Malnutrition among school age children in Africa, including Tanzania has been linked with consumption of diets lacking adequate quantity and quality of nutrients as well as inefficient utilization of available nutrients due to infections, inflammations and parasitic infestations. Currently, there is paucity of data on nutrition and micronutrient intake and status of school children in Tanzania. The present study aimed at generating data on nutrition, micronutrient and inflammation status; identified factors influencing nutrition and micronutrient status of school children of age between 5 and 12 years living in rural households of Kilosa and Chamwino districts in Tanzania.

The study also determined the micronutrient composition of selected indigenous leafy vegetables commonly consumed in the areas to examine their potential to improving micronutrient intake hence status in school children. In addition, an integrated home gardening intervention was implemented to impart skills on vegetable gardening, increase availability and intake of indigenous leafy vegetables throughout the year with the aim of reducing deficiencies of iron, vitamin A and Zinc. The study has shown that school children exhibited concurrent deficiencies of micronutrients and stunting that were strongly linked to dietary habits and infections.

Malnutrition status of school children in rural areas was of public health significance. There was a high prevalence of stunting and conditions associated with micronutrient malnutrition such as iron deficiency anaemia, vitamin A deficiency and zinc deficiency. The main factor influencing this situation is inadequate consumption (quantity and quality)

of micronutrient rich foods. Nevertheless, there was differential prevalence of malnutrition as well as factors contributing to micronutrient malnutrition in the two districts.

The prevalence of stunting and anaemia was higher in children residing in study areas of Kilosa district than in those residing in study areas of Chamwino district. Similarly, factors influencing micronutrient status differed between districts. In Chamwino district anaemia was caused by inadequate dietary intake of foods rich in iron, vitamin A and zinc. In Kilosa district anaemia was associated with high inflammation depicted as elevated C - reactive protein (CRP) and α-1 glycoprotein (AGP). The lower risks of VAD and ZnD were associated with high concentrations of serum carotenoids and α- tocopherols, respectively and the lower risks of ID was directly associated with household dietary diversity scores (HDDS).

Inflammation was identified as one of the factors influencing micronutrient status and elevated CRP and or AGP predicted VAD, ID and ZnD among school children in the study areas. The type and composition of diet also influenced the micronutrient status of school children. The differential prevalence of nutrition and micronutrient status was associated with the different types of diets that were being consumed by children in the two study areas.

Indigenous leafy vegetables (ILVs) showed high potential of improving micronutrient status if consumed in adequate amounts. The amount consumed by these children is small and therefore cannot benefit from the amount of micronutrients present in the vegetables. The quantities of micronutrients present in the vegetables vary by species.

The integrated home gardening intervention increased availability and diversity of vegetables in households; hence helped to reduce the prevalence of conditions associated with micronutrients deficiencies, especially anaemia and vitamin A. There was an increased concentration of haemoglobin and serum retinol among school children. Nevertheless, the concentration could have been higher if children were given diverse diets including animal source foods in adequate amounts.

5.2 Recommendations

This study recommends the following:

1. Information about nutrition status of school children is limited, thus hinders proper design of interventions to address their nutrition challenges. It is recommended that the Ministries responsible for Education, Health and Community Development should ensure that school children are included in national nutrition surveys (Demographic Health Survey, National Nutrition Survey) to capture information about the nutrition situation of school children. Other national surveys targeting school children should include nutrition indicators to capture integrated information about school children (e.g., school malaria parasitaemia survey, Household Budget Survey).

2. District Nutrition officers in collaboration with other stakeholders like schools, communities should promote production and consumption of micronutrient dense foods to school age children in and out of schools.

3. The Ministry of Health and its partners should consider implementing integrated health and nutrition programmes to address hygiene practices and control of infections, which were found to be highly associated with micronutrient deficiencies conditions in the present study.

4. District nutrition officers and other multisectoral nutrition stakeholders should create awareness among community members about the role of micronutrient rich foods and diet diversity in reducing the high burden of micronutrient deficiencies and undernutrition. This can be done through campaigns or strategic nutrition education programmes.

5.3 Limitations of the Study

1. A single 24-h dietary recall method to assess dietary intake of the target child could not have captured habitual dietary intake and might be subjected to seasonal variations. However, the effect of season was reduced by conducting the endline survey in the same season as the baseline survey.

2. Limited access to water for watering the gardens/vegetables and infestation by insects and destruction by animals was a challenge in the implementation of home gardens. However, the training sessions on the sustainable use of water, organic and non-organic pesticides and making of fence using the locally available materials helped to address the challenges.

3. This was a follow up study where the same participants participated during baseline survey, implemented interventions and were assessed during the endline survey. In this regard it was a before and after investigation, which did not have a control group. Therefore, it is limited in establishing the causal effect relationship.

5.4 Contribution to the Body of Knowledge

This study has provided more data on the intake and status of multiple micronutrients (zinc, iron, vitamin A, carotenoids and vitamin E) for future research and nutritional assessments in school children.

The study further demonstrated that integrated home garden interventions can contribute to the increased production and consumption of vegetables in rural households. Integrated home garden interventions can therefore be an important tool to addresses the problem of micronutrient deficiencies in low-income countries, thereby contributing to the achievement of the second sustainable development goal (SDG-2) that seeks to end hunger, achieve food security and improved nutrition and promote sustainable agriculture.

5.5 Areas for Further Research

1. There is a need to determine the composition of anti-nutritional factors and the effects of post-harvest handling, storage and cooking methods to ascertain bioavailability of micronutrients mostly present in indigenous leafy vegetables.

2. A control study design should be a priority to investigate the impact of integrated food based interventions on the nutritional status of school children in Tanzania.

APPENDICES

Appendix 1: Questionnaire for baseline survey

Scaling-up nutrition: Implementing potentials of nutrition-sensitive and diversified agriculture to increase food security

Note to enumerator: The questions in this survey are designed for the mothers/women caregivers and school children. If the mother/women caregiver is not available in the household, an appointment should be made for next visit.
(Please fill in the consent form, tick if agreed and let the interviewee sign/put thumb print on the consent form)

SECTION I: BASIC AND DEMOGRAPHIC INFORMATION

Basic information				**BI**
BI01 Date of survey: _____/_____/ _____ *(DD/MM/YYYY)*	BI02a Name of interviewee _____ BI02b Status of interviewee (select appropriate) Mothe Caregive	BI03 Sex of the interviewee _____	BI04 Name of the enumerator _____	BI05 Village name _____ _
BI06 Village code 1=Tindiga 2. Mhenda 3= Chinoje 4= Mzula *Enter/select appropriate code*	BI07 Hamlet name _____	BI08 Household ID _____	BI09 Start time _____	BI 10 End time _____ _

Demographic information DI		
DM01.	Household Head Name (Decision maker)	
DM02.	Household head sex (select one)	1=Male 2=Female
DM03	Household Head Age (in complete years)	Enter age in years _____
DM04	Household mother's/caregiver's Name	_____
DM05	Household mother's/caregiver's Age	Enter age in years _____

DM06	Position of the respondent (Head of household or Not)	
DM06	Marital Status of the mother/caregiver (select one that apply)	1=Married monogamous 2=Married Polygamous 3= widowed 4=Divorced 5=Single 6=Co-habited
DM07	Which of the following statements best describes your level of literacy? (Show the written text to the interviewee to read and select the appropriate literacy status)	1=Not able to read or write 2=Can read and write to some extent 3=I can read and write
DM08	What is your education level?	1=No formal education 2=Adult education 3=Primary school 4=Secondary school 5=Diploma/ certificate 6=University
DM09	What is your source of livelihood?	1=Farmer 2=Employed in formal sector 3= Employed in informal sector (casual labour) 4=Self employed 5=House wife / mother 88=Other (specify).....
DM10	How many people live in this Household?	Enter number
DM11	What is the total number of children in this household?	Enter number
DM12	What is the number of children in the following age categories? a. 0-2 Years b.2-5 Years c.5-15 Years (school children) d.15-18 Years	Enter number ☐ ☐ ☐ ☐
DM13	What is the name of the school child (pick the youngest school child: 5-9 years)?	Write name …… ……
DM14	Sex of the child (school child) (select appropriate)	1= Male 2=Female

DM1 5	How old is she/he (school child)?	Enter date of birth ……. ……
DM1 6	How many children did you deliver? (Number of born children)	Enter number
DM1 7	Are you pregnant? *If the answer is no go to section 2 question WS01*	1= Yes 2= No
DM1 8a	***If yes***, How many months old?	Enter number of months
DM1 8b	Interviewer enter trimester	1. First trimester (Below three months) 2. 2nd trimester (>3 to 6 months) 3. 3rd trimester(> 6 months)

SECTION II: WATER, SANITATION AND HYGIENE

Water, sanitation and hygiene			**WS**
WS 01a.	What is the main source of drinking water for members of your household during the wet season? ***(Only one main source)***	1 = Protected public well 2 = Open public well 3 = Tube well/borehole 4 = Unprotected Spring 5 = Protected spring 6 = River/stream 7 = Pond/lake 8 = Dam 9 = Piped 10= Rainwater harvesting 11= Open well in yard/plot (homestead) 88= Others (Specify)	Indicate the option here
WS01b	Which source does members of your household use for drinking during dry season? *(Among the sources listed above write the corresponding number of the response)*		Indicate the option here
WS02	What is the quality of drinking water in the household? 1. Good (fresh and clean), 2. Salty 3. Turbid 4. Salty and turbid 5. Good and salty 88. Others, specify		

WS03	What time do you take from your homestead to the source of water mentioned (in question WS 01a above-walking time) during rainy season? 1. 0-5minutes 2. 6-30 minutes 3. 30 -60 minutes 4. More than 60 minutes		
	What time do you take from your homestead to the source of water mentioned (in question WS 01a above-walking time) during dry season? 1. 0-5minutes 2. 6-30 minutes 3. 30 -60 minutes 4. More than 60 minutes		
WS04	Who is responsible for fetching domestic water for your home use? 1. Adult men 2. Adult women 3. Female children 4. Male children 5. House helpers 6. Head of household 7. Mother/caregiver 8. Other		
WS05	How do you store drinking water? (Do not read the responses)	1= In a clean container or jar 2= In a covered container 3= In a clean and covered container or jar 4= other, please specify…. 99= don't know	
WS06	Do you treat your drinking water to make it safe to drink? *If the answer is no go to question WS08*	1=Yes 2=No 99=Don't know	
WS07	***If yes***, what do you normally do?	1= boil it 2= add bleach/chlorine (Water guard) 3= sieve using a cloth 4= use a water filter (ceramic, sand, composite, etc.) 5= let it stand and settle 99= don't know 88= other, please specify	

WS08	Where do you dispose off excreta in your household?	1=Bush/open field 2=Communal pit latrine 3=VIP 4=Private toilet/latrine 5=Flush toilet 6= River 88= other, please specify	
	Is there an unused toilet?	0=No 1=Yes	
WS09	Do you clean/wash your hands after using the toilet/defecation? *If the answer is no go to question WS011*	0=No 1=Yes	
WS10	***If yes***, how do you clean/wash your hands	1= water only 2= water and soap 3= alcohol 88= others, please specify……	
WS11	Do you wash your hands before eating? *If the answer is no go to question WS10*	1= Yes 2= No	
WS12	***If yes***, how do you clean/wash your hands	1= water 2= water and soap 3= alcohol 88= others, please specify……..	
WS13	Do you wash your hands before eating?	1= Yes 2= No	
WS14	Do you think it would have some health benefits to wash hands before eating? *If the answer is no go to question WS13*	1= Yes 2= No	
WS15	***If yes***, why do you think there are some health benefits?	1= Make my hands clean 2= To protect from diseases 99= don't know 88= other, please specify …..	
WS16	Do you wash your hands after eating?	1= Yes 2= No	
WS17	Do you wash fruits before cooking/eating?	1= Yes 2= No	
	Do you wash vegetables before cooking/eating?	1= Yes 2= No	
WS18	Do you think it would have some health benefits to wash fruits and vegetables before cooking/eating?	1= Yes 2= No	

SECTION III: DISEASES HISTORY AND CONDITIONS

Diseases history and conditions			DH
DH01	Do you currently take any kind of medicine or supplements? *If the answer is no go to question DH03*	Mother/caregiver 1= Yes 2= No	Child 1= Yes 2= No
DH02	If yes what types of medicines or supplements are you taking (time???)	1=Anti malaria medicine 2=Antibiotics 3=Deworming medicines 4=Anti pains 4=Folic acid supplements 5=Iron supplements 6=Multivitamins 7=Traditional herbs 88=Others specify	*Circle where appropriate. Multiple responses allowed.*
DH03	Do you smoke or chew tobacco?	0=No 1= Yes, I smoke 2= Yes, I chew tobacco 3=Yes I smoke and chew tobacco	
DH04	Did you or the child suffer from diarrhea (define) or stomach problems in the last 4 weeks?	Caregiver 1= Yes 2= No	Child 1= Yes 2= No
DH05	Did you suffer from malaria within the last 3month?follow up question how many times *If the answer is no go to question DH 08*	1= Yes 2= No	1= Yes 2= No
DH06	If yes, how many times Did you suffer from malaria within the last 3month?	Indicate number of times in the past 3 months	
DH07	***If yes***, where do you normally get treated?	Mother 0=No treatment 1= health facility 2=hospital 3=Dispensary 4=Chemist shop 5=Traditional healers 88=Others specify……..	Child 0=No treatment 1= health facility 2=hospital 3=Dispensary 4=Chemist shop 5=Traditional healers 88=Others specify……
DH08	In general, did you visit the local health facility within the last 90 days for other reasons?	1= diarrhea 2= pneumonia 3= typhus 4= cholera 5= deworming 6= injury 88= other, specify……	1= diarrhea 2= pneumonia 3= typhus 4= cholera 5= deworming 6= injury 88= other, specify ……
DH09	Could you afford the costs of the treatment(s)? (Health facilities or hospital) *If the answer is yes go to question DH 11*	1= Yes 2= No	1= Yes 2= No
DH10	***If no***, do you have any strategies to solve the	1= ask family for money	1= ask family for

	problems to get the treatment?	2= ask local community for money 3= community health fund 4= sell livestock 5=sell crops 6= sell land 7= receive no treatment 88= others, please specify	money 2= ask local community for money 3= community health fund 4= sell livestock 5= sell land 6= receive no treatment 88= others, specify
DH11	Is there any seasonal difference in suffering from specific diseases in your household? *If the answer is no go to question HG 01*	1= Yes 2= No	
DH12	***If yes***, which disease is more frequent in your household during the dry season?	1= diarrhea 2= pneumonia 3= typhus 4= cholera 5= Worm infestation 6= injury 88= other, specify……	1= diarrhea 2= pneumonia 3= typhus 4= cholera 5= Worm infestations 6= injury 88= other, specify…
DH13	***If yes***, which disease is more frequent in your household during the rainy season?	1= diarrhea 2= pneumonia 3= typhus 4= cholera 5= Worm infestation 6= injury 88= other, specify……	1= diarrhea 2= pneumonia 3= typhus 4= cholera 5= Worm infestation 6= injury 88= other, specify…

SECTION IV: HOME GARDENING

Home gardening			**HG**
HG 01	What fruits and vegetables grow in this village or are collected from the wild? (write down all the responses)	Fruits	Vegetable
HG 02	Do you grow any of these? If 'no' go to question HG 05	1= Yes 2= No	
HG 03	Mention the types of fruits that you grow and how you do it. *Specify different fruits grown and methods used*	Fruits	Methods
			a. Flat bed b. Pocket bag c. Tray d. Container e. Intercropped in crop field
HG 04	Mention the types vegetables that you grow and how you do it. *Specify different vegetables grown and methods used*	Vegetables	Methods
			a. Flat bed b. Pocket bag c. Tray d. Container e. Intercropped in crop field

			f. collected from the wild
HG 05	Are you aware of bag/pocket gardens? If 'no' go to question section V question number GN 01	1= Yes 2= No	
HG 06	*If yes*, have you ever cultivated vegetables in a pocket garden?	1= Yes 2= No	
HG 07	What do you do with the produced fruits and vegetables *Select appropriate answer*	1=Mainly for consumption 2=Mainly selling 3=Only for selling 4=About equally consumption and selling 5=Give to neighbours, relatives 6= Donate	
HG 08	Are these vegetables given to children in your household?	1=Yes 2= No	
	Are these fruits given to children in your household?	1=Yes 2= No	
HG 09	If the answer in question HG 08 is no, give reasons	Indicate reasons here	
HG 10	Who is the main consumer of vegetables in your household?	1=Mother, 2=Father 3=Both mother and Father 4=In-laws 5=Children 6=All members, 7=the elderly 8=The sick, 9= Others specify)	
HG 11	Who is the main consumer of fruits in your household?	1=Mother, 2=Father 3=Both mother and Father 4=In-laws 5=Children 6=All members, 7=the elderly 8=The sick, 9= Others specify)	

SECTION V: IRON DEFICIENCY AND ANAEMIA

IRON DEFICIENCY		**ID**	
ID 01	Have you ever heard about anaemia? *If 'no' go to sub-section D question FP 01*	1= Yes 2= No	
ID 02	If Yes: Can you tell me how you can recognize someone who has anaemia? (Multiple responses allowed)	1= Less energy/weakness 2= Paleness/pallor 3= Spoon nails/bent nails 4= More likely to become sick (less Immunity to infections) 88= Other 99= Don't know	
ID 03	In your opinion, what do you think causes anaemia? (Multiple responses allowed)	1=Lack of iron in diet 2=Inability to absorb iron 3=Blood loss 4=Do not know 88=Other (specify) ……	

ID 04	Can you list examples of foods rich in iron?	1= Organ meat 2=Flesh meat 3= Dark green leafy vegetables 4= Beans 5=Insects (eg. grasshoppers) 6= Fish and sea foods 88= Other 99= Don't know	
ID 05	Do you know any foods that when taken during meals, help the body absorb and use iron? *If 'no' go to question ID 07*	1= Yes 2= No	
ID 06	If yes, What are those foods?	1= Vitamin-C-rich foods, such as fresh citrus fruits (orange, lemons, etc.), tomatoes, leafy vegetables 88= Other (specify)……. 99= Don't know	
ID 07	Do you know any foods that when taken during meals, decrease iron absorption? *If 'no' go to question ID 10*	1= Yes 2= No	
ID 08	If yes, What are those foods?	1= Coffee 2= Tea 88= Other (specify)…… 99= Don't know	
ID 09	In the last 6 months have you or (Name of the school child) ever used iron tablets?	**Mother/Caregiver** 1= Yes 2= No	**Child** 1= Yes 2= No
ID 10	Have you or your child received any deworming medication in the past 90 days? (Reference child is school child)	1= Yes 2= No	1= Yes 2= No
ID 11	**If yes,** what was the last time you and your child used deworming medication	Enter date, month and year	Enter date, month and year
Food Production, distribution and Consumption in the household			**FC**
FC 01	What food crops grow best in your area?	1=Cereals, roots, tubers and plantains 2=Fruits and Vegetables 3=Legumes and nuts 88=Other (specify) ……	
FC 02	Who decide what to produce in the household?	1=Head of Household 2= wife/husband 3=all members 88=Other (specify) ……	
FC 03	What types of food crops your household produces?	1=Cereals, roots, tubers and plantains 2=Fruits and Vegetables 3=Legumes and nuts 88=Other (specify) ……	
FC 04	What do you do with the produced food crops?	1=Mainly for consumption 2=Mainly selling 3=About equally consumption and selling 88=Other uses (specify)	

FC 05	Generally how many food groups are included in your typical diet?	1=2 2=3 3=4 4=5 5=More than 5 groups	
FC 06	What factors influence your consumption of different foods in a meal? *(Circle appropriate answer, multiple responses allowed)*	1=Availability 2=Affordability 3=Knowledge 4=Accessibility 5=Preparation time 6=Taste 88=Other (specify)	
FC 07	Who decide what to be cooked in the household?	1=Wife 2=Husband 3=Both wife and Husband 4=In-laws 5=Children 6=All members	
FC 08	Do household keep any type of livestock?	1=Yes 2=No	
FC 09	If yes what types of livestock do you keep? *(Circle appropriate answer, multiple responses allowed)*	1= Sheep 2=Goat 3=Cattle 4=Small ruminants (e.g rabbit) 4= chicken 5=Duck 6=Pigeon 88=Other specify	
FC 10	Who own the livestock in your household *If it is multiple responses, specify the type of livestock and indicate who own e.g husband, wife, children*	1=Wife 2=Husband 3=Both wife and Husband 4=In-laws 5=Children 6=All members	
FC 11	Who decided which livestock to keep?	1=Head of Household 2= wife/husband 3=all members 4=Child 88=Other (specify) ……	
FC 12	What are the main reasons for keeping these livestock?	1=Mainly for consumption 2=Mainly selling 3=About equally consumption and selling 4=Mechanization & animal traction 88=Other uses (specify)	
FC 13	Could you describe how animal food is distributed in your households based on different group of people e.g children, adults, head of household, caregivers *Probe for intra-household food distribution e.g who is getting what*		

FC 14	Could you describe how animal food is distributed in your households based on different groups of people e.g children, adults, head of household, caregivers *Probe for intra-household food distribution e.g who is getting what*	Head of the household	Mother	Children	Other Adults
FC 15	Give reasons as to why food is distributed that way (specify reasons based on different members of households				

SECTION VI: DIETARY ASSESSMENT AND MEASUREMENT OF NUTRITION STATUS

I. A 24 hour dietary recall- mother/woman caregiver responsible for food preparation

*Note to Enumerator: **Explanation to interviewee:** Please describe everything that you ate and drank yesterday during the day or night, whether at home or outside the home. Record the amount on the 24-hour recall sheet using household equipment. Be sure to probe until the respondent says nothing else. If the respondent mentions mixed dishes like porridge, sauce or stew, probe what ingredients were in that mixed dish.* **DR**						
Was the reference day :1= normal day (usual intake) 2= special day (festival, funeral)						
Meal time	Type of Meal	Description /ingredients of food eaten	Amount served (household measure)	Amount consumed (household measure)	Net grams served	Net gram consumed
B/F						
Snacks						
Lunch						
Snacks						
Dinner						
Snacks						

II. Food Frequency Questionnaire

Ask for frequency of consumption of different foods available in the community. Take note of where does food come from (major source) and seasonal variations **FF 01**					
Food item	Frequency of consumption			Major source	Seasonal variation

	Per day	Per week	Per Month	Rare	Never	e.g. own production, Purchase etc.	Rainy season	Dry season
Cereals								
Maize								
Sorghum								
Finger millet								
Bulrush millet								
Wheat								
Rice								
Roots, tubers, plantain								
Cassava								
Sweet potatoes								
Round potatoes								
Yams								
Green banana								
Legumes								
Beans								
Peas (Njegere)								
Cowpeas (kunde)								
Pigeon peas (Mbaazi)								
Green grams (Choroko)								
Chickpeas (Dengu)								
Soybeans (soya)								
Bambara nuts (Njugumawe)								
Lablab bean (fiwi)								
Nuts and seeds								
Groundnuts								
Coconut								
Cashew nut								
Other seeds								
Meat poultry fish and egg								
Cow-beef								
Liver								
Other organ meats								
Goat								
Sheep-lamb								
Pork								
Wild game meat								
Poultry-chicken/duck								
Eggs								
Fresh-water fish								
Sea fish								
Dried fish								
Sardines								
Sea foods								

Milk and milk products								
Cow's milk (whole)								
Goat's milk (whole)								
Processed & packed milk								
Yoghurt								
Butter/lard								
Ghee								
Cheese								
Oil and Fat								
Sunflower oil								
Red palm oil								
Korie oil								
Ground nut oil								
Other oil or fat (mention)								
Vegetables								
Cabbage								
Amaranth leaves								
Sweet potato leaves								
Cassava leaves								
Pumpkin leaves								
Carrots								
Pumpkin fruit								
Tomatoes								
Spinach								
Chinese cabbage								
African eggplant								
Eggplant								
Cowpea leaves								
Mlenda								
Other indigenous vegetables								
Fruits								
Citrus e.g oranges								
Mangoes								
Passion fruit								
Water melon								
Bananas								
Pineapple								
Papaya								
Avocado								
baobab								
Ukwaju								
Other indigenous fruits								
Beverages								
Coffee								
Tea								
Juice								
Milk								

Local brew								
Beer								
Soda								
Other beverages (mention)								

III. Anthropometric and Biochemical measurements

Anthropometric assessment and Biochemical measurements **AB 01**

This section involves measurements of nutrition status of mothers and their children (A reference child is a school child)
The protocol for conducting these measurements is attached in a separate sheet

(For weight measurements record weight in kilograms to the nearest one decimal place e.g. 14.5 kg)
(For height measurements record height in centimetres to the nearest one decimal place e.g. 24.6 cm)
(For haemoglobin measurements record readings in gram per decilitre to the nearest one decimal place e.g 12.5g/dL)

H/H No.	Sex	Age (enter DOB) (dd/mm/yy)	Age (Months /years)	Weight (kg)	Height (cm)	MUAC (cm)	Haemoglobin reading (g/dL)

Appendix 2: Sample size calculation

Sample size (n) in this study was determined as described by Fisher *et al*., (1991).

Fisher formula: $n=z^2pq/d^2$ Where:

n = the desired sample

z= standard normal deviate value (set at 1.96 corresponding to the 95% confidence interval)

d = margin error, the degree of accuracy (taken to be 5% in this study)

q =1-p

p= estimated prevalence or the proportion in the target population (taken to be 30% in this study representing any form of anaemia among women 15-49 years in Dodoma region (TDHS-MIS-2015/2016).

$n=z^2pq/d^2$

$n=1.96^2 * 0.3(1-0.3)/0.05^2$

n= 322. 69 (adding 3.5% attrition)

n=334.3 Households

Based on Scale-N project decision, the obtained sample size was doubled to accommodate both regions (Dodoma and Morogoro). Therefore a total of 669 households were sampled to participate in this study.

Appendix 3: Description of the study to participants

Introduction

My name is Victoria Gowele a PhD student from Sokoine University of Agriculture (SUA), Tanzania. As part of the requirements towards the fulfillment of my PhD Degree, I am required to carry out research. I am going to give you information and invite you and your household to participate in this research. You are welcome to ask questions whenever you feel that you need clarifications.

Aim of this study

The study is trying to examine factors affecting household dietary diversity. The researchers hope to establish the current dietary practices and influences of different factors in the context of farm production. In this research we will ask you questions related to basic nutrition knowledge, key dietary practices, food consumption pattern, and food production practices. The interview is voluntary; your participation in this study will take about 50 minutes to 1 hour.

Study population

This study will involve a total of 669 households with school children 5-10 years in Kilosa and Chamwino districts.

Benefits of participating in the study

There will be no direct benefits to you. The study will contribute to understanding on how micronutrients have a role in growth and the high rates of malnutrition specifically stunting in our country. This information will therefore be used to emphasize on importance of these nutrients at an early age.

Confidentiality

The results of your measurements and information that you give will be treated as confidential and no names will be disclosed in the reports. During data collection, your information will be linked to your name, however once the data is collected it will be coded and the information will be kept without identity of names.

Who to contact?

For further details regarding this study, you may use the following mobile phone numbers to contact the researcher (Victoria Gowele) on number 0719622501 or the research supervisors (Prof. Joyce Kinabo) on number 0754439324 and (Dr. Theresia Jumbe) on number 0754804010. Alternatively, you can contact us through the following address; Sokoine University of Agriculture, Department of Food Technology, Nutrition and Consumer Sciences. P.O. Box 3006, Morogoro, Tanzania.

Costs and compensation

You will bear no cost by choosing to participate in this study. However, a small token will be given to you as an appreciation and compensation for your time and voluntary participation.

Appendix 4: Consent form

I (Caregiver/mother's name) ……..…have been invited to participate in this research

1. I declare that I have read/ have heard and understood the research objectives

2. I have asked all questions related to the research and I am satisfied with the answers

3. I understand that any information about my household and family members will be treated and kept with required confidentiality

4. I understand that I am participating in this research voluntarily and that I can decide to answer or not answer some of the research questions and that at any given time I can decide not to continue participating in this research

5. I am ready to continue participating in further research and that if I am required to do so I will receive enough information, and any of my questions will be answered before I choose to participate

The signature below means that I voluntarily agree to participate in this research study.

Signature and/or thumbprint mother/caregiver **Date**

Appendix 5: Data collection clearance from NIMR

THE UNITED REPUBLIC OF TANZANIA

National Institute for Medical Research
3 Barack Obama Drive
P.O. Box 965
11101 Dar es Salaam
Tel: 255 22 2121400
Fax: 255 22 2121360
E-mail: headquarters@nimr.or.tz

Ministry of Health, Community
Development Gender, Elderly & Children
6 Samora Machel Avenue
P.O. Box 9083
11478 Dar es Salaam
Tel: 255 22 2120262-7
Fax: 255 22 2110986

NIMR/HQ/R.8a/Vol. IX/2226

20th June 2016

Prof Joyce Kinabo
Sokoine University of Agriculture
Faculty of Agriculture,
Department of Food Science &Technology
P O Box 3006 MOROGORO

CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Nutritional Status Analysis of Target Groups: Implementing Nutritional Education and Kitchen Gardening in Rural Tanzania for the Trans-SEC/Scale N, (Kinabo J *et al*), has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine. NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Site: Kilosa District and Chamwino District..

Approval is for one year: 20th June 2016 to 19th June 2017.

Name: Dr Mwelecele N Malecela

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY &CHILDREN

CC: **RMO**
DED
DMO

THE UNITED REPUBLIC OF TANZANIA

National Institute for Medical Research
3 Barack Obama Drive
P.O. Box 9653
11101 Dar es Salaam
Tel: 255 22 2121400
Fax: 255 22 2121360
E-mail: headquarters@nimr.or.tz

Ministry of Health, Community Development, Gender, Elderly & Children
University of Dodoma, Faculty of Arts and Social Sciences
Building No. 11
P.O. Box 743
40478 Dodoma

NIMR/HQ/R.8c/Vol. II /995

08th May 2018

Prof Joyce Kinabo
Department of Food Science
Faculty of Agriculture
Sokoine University of Agriculture
P.O. Box 3006
Morogoro

RE: APPROVAL FOR EXTENSION OF ETHICAL CLEARANCE

This letter is to confirm that your application for extension on the already approved proposal: Nutritional status analysis of target groups: Implementing nutritional education and kitchen gardens in rural Tanzania (Kinabo, J.*et al.*) has been approved.

The extension approval is based on the progress report dated 23rd April 2018 on the project, Ref. (NIMR/HQ/R.8a/Vol. IX/2226, dated 20th June 2016. Extension approval is valid until 19th June 2019.

The Principal Investigator must ensure that other conditions of approval remain as per ethical clearance letter. The PI should ensure that progress and final reports are submitted in a timely manner.

Name: Prof. Yunus Daud Mgaya

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY & CHILDREN

Appendix 6: Pocket gardening training flier

Scale-N

BUSTANI KIROBA

A. MAHITAJI

1. Kiroba chenye ujazo wa debe saba.
2. Udongo usio wa kichanga debe sita na mchanga debe tatu.
3. Mbolea ya samadi (ng'ombe, mbuzi au kondoo) iliyooza vizuri debe mbili.
4. Kokoto debe moja na nusu.
5. Mti ulionyooka wenye urefu wa mita mbili au sentimeta 200.
6. Unaweza kupima urefu wa mti kwa kulinganisha kiroba na mti kisha ongeza urefu wa hatua mbili.
7. Panga la kukatia mti na kuchimbia shimo.
8. Koleo la kuchanganyia udongo.
9. Ndoo kubwa ya kupimia udongo, mchanga na mbolea.
10. Maji ya kutosha.
11. Jembe.
12. Mbegu.
13. Kisu kikali cha kutobolea matundu ya kiroba.
14. Sadolini iliyo wazi juu na chini.

B. UTAYARISHAJI

1. Pima debe sita za udongo, debe tatu za mchanga na debe mbili za mbolea kisha changanya vizuri kwa pamoja.
2. Lowanisha mchanganyiko wa udongo, mbolea na mchanga kwa kiasi usiwe tope.
3. Hakikisha umetengeneza na kusawazisha sehemu unayoweka kiroba chako.
4. Chimba shimo kwa ajili ya kusimamisha mti wa kiroba.
5. Funga sehemu ya chini ya mti kwa kutumia mfuko wa plastiki.
6. Weka majivu kwenye shimo kuzuia mchwa kisha fukia sehemu ya chini ya mti (iliyofungwa plastiki).
7. Kunja kiroba katikati na ukate kidogo kisha valisha kwenye mti.
8. Pitisha sadolini katikati ya mti na kiroba.
9. Weka kokoto ndani ya sadolini mpaka ijae.
10. Jaza mchanganyiko wa udongo pembezoni mwa sadolini yenye kokoto iliyo ndani ya kiroba.
11. Shindilia kiasi na hakikisha kona zote zinajaa udongo kwa uwiano ulio sawa mpaka kujaa.
12. Toa sadolini kwa kuchekecha ili kokoto ziweze kujipanga vizuri ndani ya kiroba.
13. Endelea kujaza kokoto na udongo hadi kiroba kijae mpaka juu.
14. Kiroba kikijaa kimwagilie maji kwenye kokoto zinazoonekana juu ya kiroba.
15. Hakikisha sehemu zote za kiroba zinapitisha maji.
16. Toboa matundu kwa mstari ukiacha sentiminta 15 kati ya tundu moja na jingine (waweza pima kwa kutumia vidole).
17. Panda mbegu kwenye matundu uliyoyatoboa.
18. Weka uzio kuzunguka bustani yako kuzuia wanyama waharibifu.
19. Kutegemeana na unyevu wa kiroba, mwagilia walau mara mbili kwa wiki.
20. Mbegu huanza kuota kati ya siku tatu hadi saba.
21. Mboga hukomaa na kuwa tayari kuvunwa kati ya siku 21-28.

Shukurani

Utafiti umefadhiliwa na mradi wa Scale-N wa Chuo Kikuu cha Sokoine cha Kilimo (SUA) na Chuo Kikuu cha Hohenheim-Ujerumani.

Appendix 7: Steps in making a sack or pocket garden

1. Dig a hole about 20 cm deep; add ash to prevent termites.

2. Insert the rod into a hole.

3. Prepare the soil mixture (mixing soil, manure and sand).

4. Mix soil, manure, sand and water.

5. Set up the pocket garden.

6. Fill the pocket garden with soil and pebbles.

7. Water the pocket garden through the central wall of pebbles.

8. Pierce holes with a sharp knife 15 centimetres apart from each other.

9. Plant a pocket garden.

10. A pocket garden ready for harvesting.