PREVALENCE OF ZINC DEFICIENCY AMONG GHANAIAN ADOLESCENTS VERSUS FOOD COMPONENTS OF ZINC AND PHYTATE

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DECLARATION

I declare that this dissertation is the result of my own research work carried out in the Department of Nutrition and Food Science, University of Ghana, under the supervision of Professor Ebenezer Asibey-Berko of the Department of Nutrition and Food Science.

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DEDICATION

To **Raymond Akongburo Atuguba** and **Sebastian Gavor**

for the moral support through the period of my post graduate studies

and to my siblings,

Genevieve, Eadbert, Georgina and Emmanuel

whose constant support and encouragement never cease to sustain me.

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ABSTRACT

Zinc is an essential micronutrient found in almost every cell in the body. Nutrient needs are highest during adolescence, surpassed only by needs during pregnancy and lactation. The aim of this study was to determine the prevalence of zinc deficiency among adolescents in Ghana and to relate this with dietary intake of zinc and phytate. A total of 300 adolescents between ages 13-19 years were recruited from the Greater Accra and Upper East Regions for the study. Questionnaires were used for background data collection as well as food frequency intake. Anthropometric measurements were taken and plasma zinc assessed. A 46% overall prevalence of zinc deficiency (i.e. Plasma zinc <80µg/dL or <12.3µmol/L) was detected with southern subjects having a significantly higher prevalence (51%) than northern subjects (42%), ($P \leq 0.05$). Mean plasma zinc concentration detected was 81.42± 26.84 for the south and 82.96 ± 16.27 for the north. Males also had a higher prevalence of zinc deficiency than females though this was not statistically significant ($p<0.05$). The highest zinc – containing foods among the most frequently consumed foods in the north were dry fish (11.2mg/100g), groundnuts (4.5-4.7mg/100g), millet (4.7mg/100g), whole maize (3.3mg/100g) and unpolished rice (2mg/100g) and for the south, they were fish (10.9-11.3mg/100g), beef (4.1mg/100g), milk (3mg/100g), poultry (2.8mg/100g) and maize (2.4mg/100g). Highest phytate containing foods among the most frequently consumed foods were groundnut (546.1-

622.5mg/100g), maize (211.5-608.3mg/100g), millet (587.5mg/100g), unpolished rice (219.7mg/100g), and green leafy vegetables (146.5mg/100g) for the north and maize (615mg/100g), cowpea (600mg/100g), and groundnut (540mg/100g) for the south. The level of zinc deficiency found in this cohort clearly indicates that zinc deficiency is a public health problem in Ghana especially among adolescents. The possible contribution of dietary factors like low intake of zinc and its anti-nutritional factors merits a proper and broader look as well. An intervention by policy makers in the country may be necessary.

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Zinc was shown to be an essential nutrient in the mid-1930's (Todd et al, 1934). It was not until 30yrs later, however, that zinc deficiency was identified as the underlying cause of stunted growth and delayed sexual maturation in adolescent boys in Western Asia and the Middle East (Prasad et al, 1963). Zinc deficiency is now known to occur in many population groups in developing countries, and is increasingly felt to be an important public health problem (Hambidge, 1997).

The body contains approximately 2 3g of zinc, found mainly in bones, teeth, muscle, liver, kidney, hair, skin, leukocytes and testes (Sandstead, 1991). It is found in almost every cell in the body and is contained within more than 200 enzymes; substances needed for biochemical reactions. Zinc is important for a healthy immune system, for healing cuts and wounds, and for maintaining the sense of taste and smell. It also supports normal growth and development during pregnancy, childhood and adolescence (Wisconsin, 2001).

The efficiency of zinc absorption is influenced by the total content in the diet, an individual's zinc status, and the bioavailability of zinc from the diet's food components. Discounting the effect of zinc status, zinc absorption is determined largely by its solubility in the intestinal lumen, which in turn is affected by the chemical form of zinc and the presence of specific inhibitors and enhancers of absorption. The major inhibitor of zinc absorption is myoinositol hexaphosphate (phytate), which is present in many plant foods especially cereals and legumes, and irreversibly binds zinc under conditions present in the intestinal lumen.

Zinc deficiency can occur when zinc intake is inadequate, when there are increased losses of zinc from the body, or when the body's requirement for zinc increases (Wisconsin, 2001). Nutrient interactions resulting in competitive absorption can also reduce zinc intake and promote deficiency. The signs and symptoms of zinc deficiency include anorexia, growth retardation, delayed sexual maturation, hypogonadism, hypospermia, alopecia, immune disorders, dermatitis, night blindness, impaired taste (hypogeusia), and impaired wound healing (Haas, 2001).

1.2 Rationale

Adolescence is the period of transition from childhood to adulthood. It is a relatively short stage in the life cycle characterised by dramatically accelerated physical, biochemical and emotional development (Guthrie,

1989). It begins with puberty, the period when the child begins to show signs of sexual maturation. Puberty occurs commonly between ages 13 and 16 for boys. Girls enter puberty earlier than boys, usually between 11 and 13. A few girls may commence at 10 or after 13. Adolescence influences both nutritional needs and the absorption and utilization of nutrients. During this period, there is a rapid enlargement of organs and tissues, and sexual maturity brings about changes in physiological functions in response to hormonal changes. This phase of the life cycle has some of the highest nutritional needs for the males and for females and is surpassed only by needs during pregnancy and lactation.

In Ghana –a developing country- the high nutritional needs of adolescents are hardly met due to the low economic situation in the country. In addition, the diet is predominantly plant-based. Many cereals (such as maize, sorghum, millet, rice etc), vegetables (green leafy vegetables, tomatoes, pepper, garden eggs etc) and legumes (such as peas, beans and groundnuts) are consumed especially in the northern and southern parts of the country. These foods are high in phytate and fibre, which are known to be potent inhibitors of zinc absorption (Guthrie, 1989). The adolescents, with higher requirements, may therefore be lacking in this nutrient.

The worldwide prevalence of zinc deficiency is unknown. Mild and moderate forms are likely to be widespread and have until recently been largely overlooked (W.H.O., 1996). Currently, in Ghana, no study has been

conducted on the prevalence of zinc deficiency among adolescents. The essentiality of this nutrient makes it imperative for such a study to be conducted.

1.3 Main objective of study

The study therefore seeks to determine the prevalence of zinc deficiency among adolescents in Ghana and to relate these with their dietary intake of zinc and phytate.

1.4 Specific Objectives

The specific objectives of this study are as follows:

1. To determine plasma zinc levels of Ghanaian adolescents from Northern and Southern Ghana;
2. To determine and compare the prevalence of zinc deficiency by sex (male vs. female) and locale (North vs. South);
3. To determine food frequency intake and hence the most commonly consumed foods by locale (North and South);
4. To determine the zinc and phytate content of most frequently consumed foods;
5. To relate zinc status with dietary zinc and Phytate.

CHAPTER TWO

LITERATURE REVIEW

2.1 Zinc and health

Reproductive functions that have been examined in relation to zinc status are the duration of pregnancy, (including rates of spontaneous abortion); foetal growth; the timing, sequencing and efficiency of labour and delivery; and the incidence of stillbirths and congenital malformations (Brown *et al.,* 1989). Although there are clear relationships between zinc nutrition and each of these outcomes in animal models (Hurley and Baly, 1982) especially when zinc deficiency is severe, the results of human studies have been less consistent, possibly because of small sample sizes, other inadequacies in study design, and the difficulty in accurately classifying an individual's zinc status (Keen and Hurley, 1989).

Studies have also shown that there is a clear relationship between induced zinc deficiency and detrimental outcomes for each of the above aspects of animal reproductive function (Apgar, 1985). The results have not been so consistent in human studies though suggestive evidence from observational and intervention trials indicate that maternal zinc nutriture can influence several aspects of reproductive function and pregnancy outcome.

A small clinical trial was carried out in 56 British women who were thought to be at risk of delivering infants with intrauterine growth retardation because of low body weight, smoking, Asian origin, or previous delivery of a baby with this condition (Simmer *et al.,* 1991). Zinc supplemented women had significantly lower rates of induced labour (13% vs. 50%), caesarean section (7% vs. 32%), and infants with intrauterine growth retardation (7% vs. 27%), although the small sample size and differential dropout rates weaken the inferences that can be drawn from this study (Simmer *et al.,* 1991).

2.2 Morbidity

In a pooled analysis involving seven community-based trials of continuous zinc supplementation, it was observed that, the incidence and prevalence of diarrhoea were reduced with zinc supplementation, with a pooled odds ratio (OR) of 0.82 (95% confidence interval [CI] 0.72- 0.93) and a pooled odds ratio (OR) of 0.75 (95%CI 0.63-0.83) for the effect of zinc on diarrhoeal incidence and prevalence respectively (Bhutta *et al.,* 1999). Four of the studies also provided information on incidence of pneumonia, yielding a pooled OR of 0.59 (95%CI 0.41- 0.83) for the effect of zinc. Thus continuous zinc supplementation resulted in substantial and consistent reductions in both diarrhoea and acute infections of the lower respiratory tract.

A pooled results of three trials of children with acute diarrhoea indicated that those who received zinc supplementation recovered 15% more rapidly

(95%CI 6% to 22%) than those in the control groups (Zinc Investigators' Collaborative Group, 2000). A study by Roy *et al.* (1999) in which supplemental zinc was provided for just two weeks beginning at the time of initiation of treatment for either acute or persistent diarrhoea in Bangladesh children showed that, the zinc treated children continued to have reduced prevalence of diarrhoea for 1-2 months after the supplementation were discontinued. Thus, there may be some benefit of intermittent zinc supplementation in high-risk populations.

2.3 Physical growth

Brown *et al.* (1998) conducted a meta-analysis of 25 controlled clinical trials of the effect of zinc supplementation on children's growth and found out that, overall zinc supplementation had a small, but highly significant impact on children's height increments, with an average effect size of 0.22 standard deviation (SD) units. Zinc supplementation also had a small, but highly significant impact on children's weight increments, with an average effect size of 0.26 SD units. The effect of zinc supplementation on change of weight was negatively associated with mean initial plasma zinc levels.

The effect of zinc supplementation on children's growth may be due to its direct impact on nucleic acid and protein synthesis (Fraker *et al.,* 2000) and on hormonal mediators of growth (Ninh *et al.,* 1996) or its effects on appetite (O'Dell and Reeves 1989) or risk of infection (Bhutta *et al.,* 1999).

Notably in none of these studies was there significantly reduced growth velocity or any other indication of complications associated with zinc supplementation. Impact of zinc on growth was first described in humans in adolescent population in Iran and Egypt (Prasad *et al.,* 1963). In these studies, the main features of zinc deficiency were characterized by growth retardation and failure of sexual maturation.

2.4 Neurobehavioral development

Black (1998) reviewed multiple studies in experimental animals and found out that a broad range of neurobehavioral abnormalities can occur with zinc deficiency. Studies in foetuses of zinc-deprived pregnant rats demonstrated neuronal degeneration and a reduction in brain size (Proshaska *et al.,* 1974; McKenzie *et al.,* 1975), and studies in pre-pubertal monkeys with moderate zinc restriction found lower spontaneous motor activity and reduced performance of tasks that required visual attention (Golub *et al.,* 1985; Golub *et al.,* 1996).

Aggett (1989) noted in his study that, changes in mood, loss of affect, and emotional liability were pronounced enough to serve as warning signs of early zinc deficiency in patients receiving total parenteral nutrition or children with acrodermatitis enteropathica. Henkin *et al.* (1875) induced severe acute zinc deficiency in six adults by administering histidine and observed the following progression of symptoms: anorexia, dysfunction of smell and taste,

irritability, depression, and anger. These subjects also displayed lethargy, sleepiness, and decreased ability to perform simple mental arithmetic and to interpret proverbs.

In a prospective study to compare the behaviour of zinc-supplemented infants and children with those of control subjects, Friel *et al.* (1993) found that zinc-supplemented very-low birth weight Canadian infants had higher motor development scores than controls. By contrast, subsequent studies of low-birth-weight Brazilian infants (Ashworth *et al.,* 1998) and older infants and toddlers in Guatemala (Bentley *et al.,* 1997) and India (Sazawal *et al.,* 1996) found no differences in motor development, although greater activity and responsiveness were observed with zinc supplementation.

2.5 Absorption of zinc

Zinc is absorbed through the small intestines, which also regulates whole-body zinc homeostasis through changes in both the fractional absorption of dietary zinc and the excretion of endogenous zinc in pancreatic juice and other gastrointestinal secretions (Jackson, 1989). Not much is known about the precise mechanism involved (Guthrie *et al.,* 1995). Some zinc is lost from the body through urine, menstrual flow, semen, and sloughed skin, nails, and hair, although the quantity lost through these routes is small relative to the lot through gastrointestinal excretion.

Zinc absorption is mediated by a combination of simple diffusion and the binding of zinc to specific carrier substances, which are able to take zinc along with them as they enter the cells of the intestinal wall (Guthrie *et al.,* 1995). Such carriers are thought to probably include amino acids. Once zinc gets into the intestinal cells, much of it is bound to a small metalloprotein known as the metallothionen, which has the affinity of binding a variety of cations with valency of two. Metallothionen is responsible for key aspects of the homeostatic regulation of zinc absorption. Synthesis of this metallothionen is stimulated by zinc within the cells of the intestinal wall.

Another protein known as the cystein-rich intestinal protein (CRIP) then binds to zinc within the cells and shuttles it into the blood (Guthrie *et al.,* 1995). When the zinc content of the body is high, much of the zinc remains bound to metallothionen until the intestinal cells are shed from the intestinal wall and excreted along with their zinc through the faeces. On the other hand when the zinc content is low, the zinc ions are readily transferred to CRIP and transported to the blood to become bound to other proteins in the circulation, which then carries them throughout the body.

2.6 Factors affecting zinc absorption

2.6.1 Inhibitors

Naturally occurring and synthetic constituents of the human diet can interfere with the absorption and/or the utilization of dietary zinc (Solomons, 2001). Some chemically defined constituents of plants have been identified as inhibitor candidates and these have been studied in human subjects or laboratory and farm animals. These inhibitors include specific dietary fibre components (Turnland *et al.,* 1982) and tannins (Reddy and Love, 1999).

Phytic acid (myoinositol hexaphosphate) is an inhibitor of zinc absorption in most animal and human studies (Lonnerdal, 2000). It is presumed that the mechanism of interference with absorption is intraluminal binding of zinc insoluble complexes. Zinc from animal foods such as poultry and liver is however highly available for absorption since animal foods do not contain phytate. About one half of the zinc in the average diet comes from animal sources. It is for this reason that vegetarian diets for example, are often limited in zinc quantity, quality or both (Burch *et al.,* 1975). In addition, copper and non-heme iron compete with zinc at common intestinal binding sites, and thus limit the amount of zinc that is absorbed. Heme iron is however not an inhibitor of zinc absorption (Solomons, 2001).

2.6.2 Enhancers

In theory, enhancers of zinc work either by releasing zinc from inhibitory substances or by directly assisting in the transport of the metal across the intestinal membrane. Red zinfandel wine is the only food or beverage that has been shown to enhance zinc absorption (MacDonald *et al.,* 1980). A search for a zinc-binding ligand in pancreatic secretions and breast milk in the 1970s and early 1980s proved futile (Lonnerdal, 2000).

2.6.3 Zinc Nutrition Status

Much research has shown that the zinc status of an organism is regulated and evidence for homeostatic regulation, with conservation in states of zinc scarcity and less retention in states of adequacy and excess, has been reported in rats (Weigand *et al.,* 1980). Human studies show that intestinal excretion and urinary elimination of zinc can be affected by zinc status in a similar manner (Baer *et al.,* 1984).

The nutritional needs of the host influence absorption (Solomons, 2001). It seems that in pregnancy, there is an up-regulation of zinc absorption to provide the foetus with products of conception and maternal tissues with increased zinc to support the pregnancy.

2.6.4 Host related factors

Like other nutrients, any disease that produces malabsorptive states such as inflammatory bowel disease, celiac disease, crohn's disease, protein-energy malnutrition and intestinal parasitoses affect the efficient uptake of zinc (Solomons, 1982). Acute diarrhoea is associated with net loss of zinc from the intestine (Ruz *et al.,* 1995).

Conditions that affect pancreatic sufficiency also affect absorption of zinc; consequently, a decrease in absorption of zinc from food has been demonstrated in cystic fibrosis patients. This was reversed with the addition of supplemental digestive enzyme (Solomons, 2001). Pancreatic insufficiency in cystic fibrosis is caused by progressive clogging of pancreatic ducts and functional tissue degeneration resulting in lack of normal pancreatic enzymes including trypsin, chymotrypsin and carboxypeptidase (Williams, 1993), which are essential for the release of zinc from its protein matrices in tissues of plant and animal origin (Solomons, 2001).

2.6.5 Genetic factors

Potential excessive zinc absorption and a confirmed zinc malabsorption based on genetic constitution have been reported (Solomons, 2001). For example, a familial hyperzincemia in which the members of this lineage had circulating zinc concentrations in excess of 300μg/dl has been described. The error was

attributed to abnormal circulating binding affinity rather than excessive zinc intake (Solomons, 2001).

In addition to the above, acrodermatitis enteropathica, a fatal dermatological condition characterized by skin rashes, hair loss, immune deficiencies, persistent diarrhoea and malabsorption, represents reduced intestinal zinc absorption efficiency. The genes controlling these conditions have not yet been identified (Solomons, 2001).

2.7 Recommended daily allowance of zinc

Zinc deficiency in humans is mainly due to a lack of bioavailable zinc in the diet, general malnutrition or malabsorption (Good, 1981; Prasad, 1995). Nutritional zinc requirements are difficult to determine since many dietary factors affect the bioavailability of zinc, and physiological requirements of zinc vary greatly between different groups. However, recommended daily allowances (RDA by the US National Research Council) of 5mg zinc for infants (i.e. children up to one year of age), 10mg for children aged 1-14 years, 12mg for adolescents and non-pregnant and nonlactating females, and 15mg for pregnant and lactating women and male adults appear reasonable to achieve a normal plasma zinc level of 11- 18μm (National Research Council, 1989).

Population mean dietary requirements suggested by WHO (1996) are presented by age, sex, and physiological status in Table 2.1.

Table 2.1

Recommended Population Dietary Zinc Intake to meet Normative Physiological Requirements (WHO, 1996)

Population group and age range* (years)	Recommended dietary zinc intake (mg)		
	High bioavailability**	Medium bioavailability	Low bioavailability
Infants			
0-0.5	na	na	na
0.5-1	3.3	5.6	11.1
Children			
1-3	3.3	5.5	11
3-6	3.9	6.5	12.9
6-10	4.5	7.5	15
Men			
10-12	5.6	9.3	18.7
12-15	7.3	12.1	24.3
15-18	7.8	13.1	26.2
18-60+	5.6	9.4	18.7
Women			
10-12	5	8.4	16.8
12-15	6.1	10.3	20.6
15-18	6.2	10.2	20.6
18-60+	4	6.5	13.1
Pregnant women, any age (mean for all trimesters)	6	10	20
Lactating women, any age			
0.5months after birth	7.3	12.2	24.3
>5 months after birth	5.8	9.6	19.2

Na = not applicable, because breastfed infants < 6months of age are assumed to be able to satisfy Zn requirements from human milk only.

*For each age range, subjects up to but not including the age indicated by the upper value were included. For example, the range 1-3 years includes children from exactly 1 year of age to 3 years less 1-day (i.e. does not include children exactly 3years old).

**Low bioavailability: 45% to 55% absorption of Zn intake (phytate- zinc molar ratio <5); medium bioavailability: 30% to 35% absorption of Zn intake (phytate- zinc molar ratio =5-15); low bioavailability: 10% to 15% absorption of Zn intake (phytate- zinc molar ratio >15)

Source: (Brown and Wuehler, 2000)

2.8 Dietary sources and zinc availability

The bioavailability of zinc and the total zinc content of the diet both influence the efficiency of zinc absorption (Brown and Wuehler, 2000). The bioavailability of zinc from foods varies extensively, depending on the presence of specific inhibitors (e.g. phytate) and enhancers in the food, as well as the solubility of zinc in the intestinal lumen. It is generally considered that diets with a phytate-zinc molar ratio greater than 15 have relatively poor zinc bioavailability, those with a phytate-zinc molar ratio between 5 and 15 have medium zinc bioavailability, and those with a phytate-zinc molar ratio less than 5 have relatively good zinc bioavailability (WHO, 1996).

Sandstrom recently summarized from her own and others' research (Sandstrom and Ceberblad 1980,1987; Flanagan et al. 1985; Navert et al. 1985; Sandstrom et al. 1985; Sandstrom et al. 1987; Sandstrom et al. 1989) results of previous studies of the fractional and net absorption of zinc under different dietary conditions and found out that, when zinc sulfate was consumed in aqueous solution by fasting individuals, the mean fractional absorption ranged from 46% to 74%. Although fractional absorption decreased with higher intakes of zinc, the net absorption of zinc was greater when the total consumption of zinc increased.

When the same zinc solutions were consumed with small amounts of rice crisps or wheat bran, fractional zinc absorption declined considerably, to about 5% to 40% of the original levels (resulting in final fractional absorptions of 2% to 22%). The fractional absorption of zinc was also reduced when other minerals were included in the aqueous supplement, although the decrement in fractional absorption of zinc with these additional minerals was less dramatic than with the phytate-containing foods.

Sandstrom et al. 1989, also found that, fractional absorption of zinc from milk formulas (about 32%) was substantially greater than from soy formulas (about 12%). The absorption coefficient did not change much when more zinc was added to the formulas, so fortification resulted in greater net absorption of zinc. Zinc absorption from white and whole-meal breads varied with phytate content, and fractional absorption in both types of bread decreased to less than half the original levels when they were fortified. Also, zinc was most efficiently absorbed from meals containing animal products.

The richest food sources of zinc include organs and flesh of mammals, fowl and fish. Crustaceans are the richest sources (Table 2.2). Eggs and dairy products are also free of phytate, although they have slightly lower zinc content than organ and flesh foods. Most cereals and legumes have an intermediate level of zinc, but their high phytate content reduces the amount of zinc available for absorption. When these staples are fermented (as occurs with leavened breads and with porridges prepared from fermented cereals),

the fermenting organisms produce phytases that break down the phytate, which increases the amount of absorbable zinc. Rice and starchy roots and tubers have lower zinc contents than legumes and cereals other than rice. Fruits and vegetables are generally not rich sources of zinc, although some green leafy vegetables, such as spinach, have a fairly high zinc density, albeit of uncertain bioavailability (Brown and Wuehler, 2000).

The absorption of zinc from a mixed diet can be estimated by a phytate –zinc molar ratio; calculated as follows:

$$\frac{\textit{Phytate content of foods}/660}{\textit{Zn Content of foods}/65.4}$$

Where 660 and 65.4 represent the molecular or atomic weight of phytate and zinc, respectively.

Information on phytate content of various foods has been compiled in several databases (Oberleas and Harland, 1981; Oberleas, 1983; Reddy *et al*, 1989). Generally, it is considered that diets with a phytate-zinc molar ratio greater than 15 have a relatively poor zinc bioavailability, those with a phytate-zinc molar ratio between 5 and 15 have median zinc bioavailability, and those with a phytate-zinc molar ratio less than 5 have relatively good zinc bioavailability (WHO, 1996). WHO (1996) has described the relationship between zinc intake, the phytate-zinc molar ratio of the diet, and zinc absorption.

Table 2.2

Zinc and Phytate contents of different foods, and estimated amounts of zinc available for absorption*

Food Type	Zinc Content		Phytate content		Estimated absorbable zinc
	in mg/100g	in mg/100kcal	in mg/100g	Phytate-Zn molar ratio	mg/100g
Liver, kidney (of beef or poultry)	4.2-6.1	2.7-3.8	0	0	2.1-3.1
Meat (beef, pork)	2.9-4.7	1.1-2.8	0	0	1.4-2.4
Poultry (e.g. chicken, duck)	1.8-3.0	0.6-1.4	0	0	0.9-1.5
Seafood (excluding oysters)	0.5-5.2	0.3-1.7	0	0	0.2-2.6
Eggs (chicken , duck)	1.1-1.4	0.7-0.8	0	0	0.6-0.7
Dairy products (cow milk, cheese)	0.4-3.1	0.3-1.0	0	0	0.2-1.6
Seeds, nuts (e.g. sesame, pumpkin, almond)	2.9-7.8	0.5-1.4	1760-4710	22-88	0.3-0.8
Bread (made with white flour, yeast)	0.9	0.3	30	3	0.4
Whole grain cereal (e.g. wheat, maize, brown rice)	0.5-3.2	0.4-0.9	211-618	22-53	0.1-0.3
Beans, lentils (e.g.soy, kidney bean, chickpea)	1.0-2.0	0.9-1.2	110-617	19-56	0.1-0.2
Refined cereal grains (eg. White flour, white rice)	0.4-0.8	0.2-0.4	30-439	16-54	0.1
Fermented cassava root	0.7	0.2	70	10	0.2
Tubers	0.3-0.5	0.2-0.5	93-131	26-31	<0.1-0.2
Vegetables	0.1-0.8	0.3-3.5	0-116	0-42	<0.1-0.4
Fruits	0-0.2	0-0.6	0-63	0-31	<0.1-0.2

* Amount of zinc available for absorption estimated as 45% to 55% if phytate-zinc molar ratio < 5, as 30% to 35% if phytate zinc molar ratio = 5-15, and as 10% to 15% if phytate-zinc molar ratio > 15.

Source: Brown and Wuehler, 2000

Three categories of diet were described, with high, medium or low zinc availability, based on the proportion of energy from animal sources, the type of processing of cereals, the amounts of inorganic calcium salts, and the phytate-zinc molar ratio. It was established that about 45 to 55% of the amount of dietary zinc is absorbed from a high bioavailability diet, 30 – 35% from a medium – bioavailability diet, and 10 – 15% from a low bioavailability diet depending on the zinc content of the meal (and assuming a typical intake of approximately 3-5mg zinc per meal). Further work is needed to validate the ability of dietary data to predict zinc status because of remaining uncertainties in the estimates of zinc absorption from different diets by individuals with different zinc status.

In summary, zinc is absorbed most efficiently from aqueous solutions and from foods containing animal products. Fortification of foods with exogenous zinc led to a reduction in fractional absorption, but a positive impact on net absorption. Fortification of foods with high phytate – zinc molar ratio had only a small effect on net zinc absorption (Sandstrom and Cederblad, 1980; Sandstrom *et al*, 1980; Farah *et al*, 1984; Lonnerdal *et al*, 1984; Valberg *et al*, 1984; Flanagan *et al*, 1985; Navert *et al*, 1985; Sandstrom *et al*, 1985; Sandstrom *et al*, 1987; Sandstrom *et al*, 1989).

2.9 Assessment of Zinc Nutrition Status

Several authors have reviewed the range of techniques that have been proposed for evaluating the zinc nutriture of individuals and populations (King, 1990; Aggett and Favier 1993; Hambridge and Krebs 1995). Some of these are outlined as follows:

2.9.1 Functional indices

In theory, the activities of zinc-dependent enzymes in plasma or blood cells should be useful as functional indices of zinc status. Enzymes such as lactate dehydrogenase and thymidine kinase have been studied in this respect to date; none has proven reliable as an indicator of zinc deprivation (Sandstrom, 2001).

Based on the observation in severe zinc deficiency, other functional tests such as taste acuity, leukocyte chemotaxis and cutaneous hypersensitivity have been suggested. In spite of the fact that these are not specific enough to diagnose zinc deficiency in individuals and populations, they can be used to monitor response to zinc supplementation (Sandstrom, 2001).

2.9.2 Erythrocyte zinc

Erythrocyte zinc concentration is about ten times higher in plasma. The slow turnover rate of erythrocytes means that their content cannot reflect recent changes in zinc supply, but they may be useful for studies of chronic zinc deprivation. However, a cut-off level has not been established and at the moment erythrocyte zinc is not a useful measure of zinc status (Sandstrom, 2001).

2.9.3 Leukocyte zinc

Leukocytes have shorter half-life and are thus assumed to be more sensitive to changes in zinc supply than erythrocytes. The limitation here is that the different types of white blood cells have different half-lives and different zinc concentrations and as such, analyses of zinc content of mixed leukocytes may be difficult to interpret (Sandstrom, 2001).

2.9.4 Hair zinc

Integumental and hair zinc concentrations respond to changes in zinc supply and monitoring of hair zinc content appears to be useful in determining response to increased zinc supply (Gibson *et al.,* 1989). However, increased hair zinc levels have been observed in animals and malnourished children with severe zinc deprivation (Erten *et al.,* 1978). This observation may be due to reduced hair growth rate resulting from malnourishment. A cut-off

level of 1.07mmol/g has been suggested as an indicator of low zinc status (Sandstrom, 2001).

2.9.5 Urinary zinc

A decreased excretion of zinc in urine may be an indicator of zinc deficiency. However, the pathological and metabolic state of the individual must be considered, as these have an effect on urinary zinc excretion (Sandstrom, 2001).

2.9.6 Rapidly exchangeable zinc pools

The rapid onset and disappearance of clinical zinc deficiency symptoms suggest the presence of an easily accessible pool of zinc. Though the technique is too advanced and costly for population studies, it may be used as a gold standard for establishing functional cut-off levels for more easily measured indices like plasma zinc (Sandstrom, 2001).

2.9.7 Mean plasma or serum zinc concentration

Despite the difficulties in interpreting the plasma zinc concentration of individual subjects, several pieces of evidence suggest that the mean plasma zinc concentration of a group of individuals may provide useful information on the zinc status of the population from which that sample is derived. For example, when the zinc intake of groups of volunteer study subjects is

severely restricted, the plasma zinc concentrations diminish within a fairly short period (Baer and King, 1984).

Results from a recent meta-analysis of zinc concentration indicate that the mean plasma zinc concentration of subjects in individual studies predicted the magnitude of the response in weight gain following zinc supplementation (Brown *et al.,* 1998). When the initial mean plasma zinc concentration was greater than about 80μg/dL (about 12μmol/L), there was no response to zinc supplementation. With decreasing plasma zinc concentrations, there was, in general, a progressive greater response to zinc supplementation. Plasma zinc cut-off indicating zinc deficiency for these adolescents therefore is < 80μg/dL (or < 12.3μmol/L). For pregnant women in the 2^{nd} and 3^{rd} trimesters, plasma zinc cut-off values indicative of zinc deficiency is < 50μg/dL or 7.6μmol/L (Hotz C. and Brown K.H., 2004).

Moreover, almost all studies found a significant increase in the plasma zinc concentration following supplementation, suggesting that this indicator could also be used to assess successful delivery of zinc supplements. Also, further evidence suggests that when coupled with the prevalence of stunting, a careful evaluation of plasma zinc concentration in representative samples in the population is a useful indicator of the prevalence of zinc deficiency in the population (Sandstrom, 2001).

2.10 Zinc deficiency

The extent of zinc deficiency has not been precisely quantified among populations because of lack of a simple and sensitive tool to measure zinc status. Zinc is associated with more than 50 distinct metalloenzymes, which have diverse functions such as synthesis of nucleic acids and specific proteins, such as hormones and their receptors (Brown *et al.,* 2001). About two dozens of these known metalloenzymes control fundamental metabolic processes involving nutrients. It is for this reason that zinc deficiency brings multiple problems in the dysfunction of many body systems (Williams, 1993).

The close association between zinc deficiency and increased diarrhoeal and respiratory morbidity has been demonstrated (Bhandari *et al.,* 1996; Bahl *et al.,* 1998). Several investigators have demonstrated a significant and consistent reduction in the severity and duration of diarrhoea following zinc administration particularly, when it is prolonged (Roy *et al.,* 1999; Penny *et al.,* 1999; Sazawal *et al.,* 1997a; Sazawal *et al.,* 1995) by supplementing with zinc in varying doses.

Clinical problems associated with zinc deficiency include hypogonadism, a condition in which there is dwarfism and a diminished function of the gonads; hypeguesia (impaired taste); hyposmia (impaired smell); retarded wound healing and acrodermatitis enteropathica.

Pregnant and lactating women, infants and young children have been identified as being especially at risk of zinc deficiency (Brown *et al.,* 2001). This is likely due to the fact that zinc is indispensable during periods of rapid growth both pre and postnatal and for tissues with rapid cellular differentiation and turnover (Brown *et al.,* 2001).

Observations of patients on long-term parenteral nutrition (TPN) have confirmed risks of zinc deficiency (Fleming, 1998) and trace element supplements of zinc in addition to copper are needed especially by patients with severe burns because of their essential roles in wound healing during acute recovery period (Cunningham, 1991).

2.11 Zinc toxicity

Zinc has low toxicity, although acute symptoms of nausea, vomiting, diarrhoea, fever, and lethargy may be observed when large doses (i.e. > 1g) are consumed. In cases where zinc intake exceeds physiological needs by reasonably small amounts homeostasis can be maintained by increased endogenous faecal and urinary excretion (Brown *et al.,* 2001). However, if excessive zinc intake continues for prolonged periods usually due to excessive use of zinc supplements (Forman, 1990), absorption of other trace elements especially copper and iron can be impaired (Brown *et al.,* 2001). For example, intake of supplements providing 50mg zinc per day for 6 weeks

produced changes in erythrocyte copper-zinc superoxide dismutase, an indicator of copper status (Fischer *et al.,* 1984; Yadrick *et al.,* 1989).

At higher doses of zinc (160- 660mg/d), anaemia and changes in immune function and lipoprotein metabolism were observed, in addition to abnormal indices of copper status (Porter *et al.,* 1977; Hooper *et al.,* 1980; Chandra, 1984; Patterson *et al.,* 1985).

2.12 Prevalence of zinc deficiency

There are still arguments on the likelihood of widespread zinc deficiency in low-income countries (Sandstead, 1991; Shrimpton 1993; Gibson, 1994). However, quantitative estimates of the percentage of the global population at risk of inadequate zinc nutriture and specific information on the prevalence of deficiency in particular settings are still lacking mostly due to the aforementioned difficulties in the assessment of zinc status (Brown *et al.,* 2001).

The percentage of the population at risk of zinc deficiency was estimated for each country as follows: the mean normative dietary requirement of zinc was assumed to be 50% of the WHO population dietary requirement. The availability of food sources of zinc to individuals was assumed to follow a Gaussian distribution, with the standard deviation equal to 25% of the mean (suggested by WHO, 1996). The percentage of the population at risk is

calculated as the area under the normal curve to the left of the normative physiological requirement (Brown *et al.,* 2001).

The percentage of the national population at risk of low zinc intake ranges from a low of 1% to 13% in countries of Europe and North America to a high of 68% to 95% in South and Southeast Asia, Africa, and the eastern Mediterranean region (Brown *et al.,* 2001).

Analysing previously collected information on the total daily per capita amount of zinc in the national food supply in relation to the population's theoretical zinc requirements is an indirect method of estimating global rates of zinc deficiency (Brown *et al.,* 2001). Data on the amounts of major food commodities available for human consumption are listed for most countries in the Food and Agriculture Organisation's food balance sheets (FAO, 1998).

However, this approach of estimating the prevalence of zinc deficiency has the following weaknesses: first of all, because the amounts of zinc in the national food supplies is strongly correlated with the total amount of food energy, any underestimates in the available food supply will result in overestimates of the number of individuals at risk of low intake and vice versa. Secondly, the food balance sheet does not provide information on the distribution of the food supply among and within households and thus actual variability would not be known and this tends to underestimate the percentage of individuals at risk of low intake in countries with food zinc

supplies above the requirement and overestimate the percentage of individuals at risk of low intake in countries with food zinc supplies below the requirement (Brown *et al.,* 2001). In addition, the WHO estimates of age and sex-specific normative physiological requirements assume that these are fixed when in fact they vary among individuals.

Notwithstanding the uncertainties in the estimates of the adequacy of zinc in the national food supplies, the data provide information on countries likely to be at greatest risk of low intake of absorbable zinc and consequent zinc deficiency. Notably these countries tend also to have elevated rates of infant and child mortality, low birth weight, and postnatal malnutrition (UNICEF, 1999).

CHAPTER THREE

METHODOLOGY

3.1 Sample size

The following formula was used to calculate the sample size (Selvin, S., 1991):

$$N = \frac{Z^2 \times P(100 - P)}{D^2}$$

Where:

Z is the critical probability value for the 95% confidence level

P is the proportion of subjects of interest in the population

D is the margin of error (level of precision) accepted for measuring plasma zinc.

N is the sample size

The Ghana Demographic and Health Survey in 1998 revealed that adolescents (ranging from ages 10 19) made up 23.4% of the Ghanaian population (Ghana Statistical Service, 1999). A 'D' value of 5 was chosen, meaning that the true prevalence among Ghanaian adolescent population should lie within ± 5 of the prevalence found among the study sample (F.A.O., 1990). Thus, the sample size used in this study was calculated as:

$$\text{Sample size, } N = \frac{1.96^2 \times 23.4\,(100-23.4)}{5^2}$$

$$= \frac{3.8416 \times 1792.44}{25}$$

$$= 275 \text{ subjects.}$$

Based on this calculation and including a 10% attrition or dropout rate, 300 subjects were recruited for this study.

3.2 Study design

The design used for this study was cross-sectional. This is a type of study whereby subjects are met just once and all data needed collected. In this case, each school was visited on a particular day and all the information needed was collected through questionnaires, anthropometric measurements and blood sample collection.

3.2.1 Study population

Southern subjects were recruited from the following three schools in the Ayawaso District in the Greater Accra Region:

1. Association School
2. Kanda '1'J.S.S.
3. Kokomlemle '2' J.S.S.

Northern subjects on the other hand were recruited from the following three schools in the Kasena-Nankana District in the Upper East Region:

1. Monsignor Abatey J.S.S.
2. St. Mary's J.S.S.
3. Kwarania J.S.S.

The Greater Accra Region, being the capital city of the nation, is more complex and developed than the Upper East Region. People in this region have easy access to electricity, treated water, the Internet etc than those from up north. Accra also has a good representation of all the various tribes found in Ghana due to the high incidence of rural-urban drift. Cost of living in Accra is high as compared to Navrongo and since it is geographically situated at the coast of the Gulf of Guinea, seafood (fish) is readily available at moderate prices. Because of the above reasons, the dietary patterns and lifestyles of Southerners may differ from those of Northerners.

The map of the two study sites is shown as Figure 3.1

3.2.2. Sampling procedure

A list of all schools within the Ayawaso and Kasena Nankana Districts was obtained from the Ghana Education Service. Names of all J. S.S. Schools within the Ayawaso District were written out, folded and placed within one bowl. The lists of schools were thoroughly mixed up manually and chosen schools randomly selected. All students within the age bracket of the study (13 -19years) were informed about the study and given consent forms to be filled by their parents. Names of pupils, whose parents consented to the study, were sorted and subjects subsequently selected using the stratified sampling method (Selvin, 1991). Equal number of girls and boys was aimed at.

FIG. 3.1 MAP OF TWO STUDY SITES

3.2.3 Written Consents

Permission was sought from the Ghana Education Service and from the heads of the six schools where the study was conducted (Association School, Accra; Kokomlemle 2 J.S.S., Accra; Kanda 1 J.S.S., Accra; St. Mary's J.S.S., Navrongo; Monsignor Abatey J. S. S., Navrongo; and Kwarania J.S.S., Navrongo.). Written consent (Appendix I and II) was also obtained from parents of subjects and subjects themselves after the study had been clearly explained to them.

3.2.4 Ethical considerations

In view of the fact that human subjects were to be used, ethical clearance was sought and obtained from the Institute Review Board of Noguchi Memorial Institute for Medical Research, Legon. A copy of the ethical approval certificate obtained is shown in Appendix V.

3.2.5 Questionnaire pre-testing

Pre-testing of questionnaire was conducted at Anunmle J.S.S. Thirty students (10% of total sample size) of the same age group as study subjects were allowed to fill the questionnaire to the best of their ability. Students were then offered the opportunity to state which of the questions were not clear, or were difficult to comprehend. Based on these and the results of analysis of the filled forms, the original questionnaire was readjusted to the current one used (see appendix IV).

3.2.6 Inclusion criteria

Study subjects were chosen based on the following criteria:

1. Should be between the ages of 13 and 19 years.
2. Must have no serious health impairment. This means subjects should not be confined to a bed, either by themselves or by the instruction of medical doctor.
3. They must be ambulatory and going about their business.
4. Should have lived in the region of recruitment for at least the past one year.

3.3 Field Interviewers

Field Interviewers were selected from the regions of study and criteria used in their selection included the ability to speak the local dialect, a 1st degree in a science related course with some basic knowledge in Nutrition as well as a friendly attitude towards others. Training for Interviewers was conducted prior to data collection. This involved explanation of the purpose of the study, how to administer the questionnaire and how to relate in a friendly manner to the subjects.

3.4 Data and Blood Sample Collection

3.4.1 Questionnaire Administration

Fifty subjects from each school filled the questionnaire with the assistance of interviewers. Questions were read and translated into local dialects by the interviewers to ensure comprehension of questions by subjects (Appendix VIIc). Questions were aimed at collecting information on background data of subjects including information on their socio-economic status and food frequency intake including food types and portion sizes. Samples of the various food types were shown to subjects in some cases to ensure correct identification and classification of the food. For example, samples of the different types of bread (brown bread, white bread) or cereals (maize, millet, sorghum etc) were shown to them.

Presence of clinical signs of zinc deficiency such as glossitis and loss of appetite were also checked for and filled on the questionnaire, being fully aware of the non-specificity of clinical signs.

- ❖ Quality Control

Field personnel crosschecked all filled forms for errors after subjects had completed filling the forms before returning from the field.

3.4.2 Anthropometric Measurements

- Height

The subjects stood erect and barefooted on the Harpenden Stadiometer with both feet placed in the centre of the footpad and both arms by their sides. The head was held such that the eyes of the subjects were at right angles to the Stadiometer. The readings were then taken to the nearest 0.1cm by lowering the headpiece gently on the head. Some photographs on field data collection are shown in Appendix VIII.

- Weight

For the weight measurement, subjects were asked to empty their pockets and discard all heavy clothing (such as thick belts, watches, bags, shoes etc) before mounting the Seca Weighing Scale. Subjects were made to stand erect with their feet placed centrally on the scale, their arms at their sides and head looking forward. Readings were taken to the nearest 0.1kg.

3.4.3 Plasma Sample Preparation

Blood sample (5ml) was collected from each subject by a Phlebotomist and immediately emptied into heparinized ion-free tubes. The tubes containing the blood samples were turned gently a couple of times to ensure no clotting of the blood. Plasma was separated by centrifuging within four hours of sampling and stored in a refrigerator. From the field to the laboratory,

plasma samples were transported in an Ice-Chest covered adequately by ice packs and immediately stored under freezing conditions upon reaching the laboratory.

3.4.4 Food sample collection

Food samples were obtained from two local markets within each region of the study sites. In the Greater Accra Region food samples were obtained from the Achimota Market and Madina Market; whilst in the Upper East Region, food samples were bought from the Navrongo and Paga markets. The fresh food samples purchased were washed and bagged and transported in an Ice Chest filled with ice packs to the laboratory where some were stored in the cold room and others in the refrigerator.

3.5 Laboratory analysis

3.5.1 Plasma zinc determination

Atomic Absorption Spectrophotometry was used to determine plasma zinc. A 'seronorm' standard reference material was used for quality control. Analysis was done on duplicate plasma samples.

3.5.2 Food moisture determination

The Air-Oven method (AOAC, 1975) was the procedure used.

❖ Principle

The sample is dried to a constant weight in an air oven. The difference in weight between the fresh and the dried sample is moisture content.

❖ Method

Clean and labelled moisture cans were dried to a constant weight in an Air-Oven and weighed after cooling in a dessicator. Approximately, 2g of the food sample was weighed into a moisture can and dried at 105°C overnight in the oven leaving covers slightly ajar. At this temperature, moisture was expected to evaporate from the samples. The cans were removed from the oven after first tapping the covers and closing the cans. The contents of the moisture cans were then cooled in a dessicator and weighed on a Mettler Balance till a constant weight was obtained.

❖ Calculation

$$\%\ Moisture = \frac{Weight\ of\ Moisture\ x\ 100}{Weight\ of\ Sample\ Used}$$

3.5.3 Food zinc determination

❖ Cleaning of Glassware

All glassware used in food zinc analysis were soaked overnight in a 30% solution of Versaclean, washed, rinsed well with de-ionised water and dried

before use in order to prevent any contamination that could lead to significant errors.

- Wet Digestion

The wet ashing method was used in eliminating the organic materials. Samples of 3g each of the commonly consumed foods were accurately weighed into a 250ml Erlenmeyer flask. Concentrated Nitric Acid of 75ml was added and the flask covered with a watch glass.

The sample was then digested with great care on a hot plate in a fume chamber until all the organic matter had been oxidized which usually took 20-30minutes resulting in a pale yellow solution. The solution was cooled and 3ml Perchloric Acid (70% $HCLO_4$) added with care. The digestion was then continued till a colourless or almost colourless solution resulted. In cases where the solution turned black, it was immediately removed from the heat source and cooled after which 15ml HNO_3 and 6ml $HClO_4$ was added before digestion was continued. Digestion continued until all the Nitric Acid had been removed. When digestion was complete, the solution was cooled slightly and 90ml distilled water added. This solution was then brought to boil for about 10minutes, and then filtered whilst hot through a No. 44 Whatman paper into a 100ml volumetric flask. The flask was then cooled and made up to the mark and stored in the cold room till analysis was carried out.

❖ Atomic Absorption

Wet-digested samples were analysed for zinc concentration by Atomic Absorption Spectrophotometry at the Ecological Laboratory of the Geography Department, University of Ghana, Legon.

❖ Principle

Only up to 10% of the sample aspirated into the flame gets atomised. This represents portions of the sample that pick up enough energy from the flame to be changed into atoms. In the case of zinc, electrons of these atoms will absorb much light at a wavelength of about 214.8nm. The absorption will be proportional to the zinc concentration in the sample when measured. Flame temperature (depending on the fuel being used) is chosen to maximize atomisation. The higher the flame temperature, the higher the atomisation and sensitivity (Pomeranz and Meloan, 1977). Atomic Absorption has higher sensitivity and specificity than conventional spectrophotometric and colourimetric procedures. The water used in the analysis was de-ionised, free of impurities and with a specific resistance of 18.2megohm-cm. Zinc is ubiquitous, thus extra precaution is necessary to prevent contamination from exogenous sources.

❖ Procedure

Standard solutions of 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0ug/ml, prepared from a commercial stock standard of 1000ug zinc/ml were used in calibrating the spectrophotometer before analysis of the wet digest of the food samples and quality control sample commenced. Analysis of samples was done in triplicate.

3.5.4 Phytate determination

The content of phytic acid in food samples most commonly consumed by subjects was determined by the method of Wheeler and Ferrel, 1971.

❖ Principle

This method is based essentially on the extraction of phytate with 3% trichloroacetic acid and its precipitation as ferric salt. The iron content of the precipitate is determined colorimetrically and the phytate phosphorus content calculated from this value, assuming a constant 4Fe: 6P molecular ratio in precipitate.

❖ Procedure

An aliquot (10g) of the food sample was weighed in triplicate into a 250ml Erlenmeyer flask and 50ml of 3% Trichloroacetic Acid added. The solution

was made to stand for 45mins, occasionally swirling by hand. After, the suspension was transferred into a 50ml Centrifuge tube and centrifuged for 15 minutes at 3000g. After centrifugation, 10ml of the supernatant was transferred into another 50ml Centrifuge tube and 4ml Ferric Chloride (2mg/ml) added to the aliquot by pipette.

The tube and its contents were heated in boiling water for 45minutes (and when supernatant was not clear after 30minutes, 2 drops of 3% sodium sulphate was added and heating continued) and centrifuged again for 15 minutes. After, the clear supernatant was decanted (poured away); the precipitate was washed twice by dispersing in 25ml of 3% Trichloroacetic Acid. The precipitate was then heated in boiling water bath for 10 minutes after which it was centrifuged again.

The supernatant resulting was washed once with distilled water and precipitate dispersed in 2ml distilled water and 3ml of 1.5N NaOH. 25ml of distilled water was added to bring volume to 30ml and the mixture heated in boiling water bath for 30minutes. The mixture was then filtered hot (quantitatively) through a Whatman No. 2 retentive filter paper. The precipitate was washed with 20ml hot water before being washed from the paper with 40ml hot 3.2N Nitric acid into a 100ml volumetric flask. Several portions of distilled water were used to wash the filter paper, and the washings collected in the same flask. The flask and its contents were then cooled to room temperature and diluted to volume with distilled water.

For the preparation of the standard, 0.1g of Ferric Nitrate was accurately weighed on Mettler balance and washed to mark into a 100ml volumetric flask. Portions of this solution (2ml, 4ml, 6ml, 8ml, and 10ml) were transferred into 10ml volumetric flasks and distilled water added to mark in all the volumetric flasks and shaken except that containing 10ml.

Into a 100ml volumetric flask, the following were pipetted:

Table 3.1:

Spectrophometric readings of phytate samples

	BLANK	TEST	STANDARD
1. Distilled water	5.0ml	-	-
2. Standards (2-10)	-	-	5.0ml
3. Test sample solution	-	5.0ml	-
4. Distilled water	65.0ml	65.0ml	65.0ml
Spectrophotometry Readings			
5. Potassium Thiocyanate (1.5N)	20ml	-	20ml
6. Blanks and standards were made up to 100ml, mixed and read at 480nm **within one minute**			
7. Potassium Thiocyanate (1.5N	-	20ml	-
8. Test sample was made up to 100ml with distilled water, mixed and read at 480nm			

3.6 Statistical analysis

3.6.1 Data entry

- Questionnaire

The EPI-INFO Version 6 program was used in data entry and analysis of questionnaire. The double entry procedure was used to ensure the right information was entered. This procedure involves a second entry of data by a different person after the initial data entry has been conducted. This way, wrong data entered are discovered and corrected. Data entry and analysis of plasma zinc values was by the S.P.S.S. program. Most of the data analysis on food zinc and phytate was done in Microsoft Excel.

3.6.2 Analysis

Statistical analysis conducted included

1. Frequency distributions of subjects' plasma zinc to check whether the distributions are normal or skewed.
2. Calculation of zinc and phytate contents of most commonly consumed foods. The mean ± SD or median values of these parameters were also determined.
3. Student's *t*-test was used for statistical comparisons of plasma zinc levels between Northern and Southern subjects. Statistical significance was given at $p<0.05$ level.

4. A correlation matrix was generated to determine the statistical associations between the various measurements.

CHAPTER FOUR

RESULTS

Background information on the subjects

Information on subjects analysed included demography, religion, lifestyle and socio-economic status. A more detailed map of the study area is shown on Fig 4.1 and 4.2.

- Demographic data

Most of the subjects under study were born in their respective regions of study (63% in Greater Accra Region, GAR and 89% in the Upper East Region, U.E.R.) and majority had lived in those regions for over 10years (69% and 85%, respectively for GAR and UER). Only 3% were born outside Ghana. Thus, most of the subjects recruited had lived in their respective regions of study for more than half their lives. This ensures adaptation of subjects to the areas of study.

The age range of the cohorts used in this study was 13- 19 years. Majority of the subjects were found to be between the ages of 13 and 16 years for both Northern (83%) and Southern (92%) respondents, with only 17% and 8%, respectively between ages 17-19 years. The modal age of the subjects was 14 years (29% and 31% for G.A.R. and U.E.R., respectively). Males

constituted 49% of the subjects. None of the subjects was married (Table 4.1). All subjects recruited fell within the adolescent age of 11-19 years (Family Health Guide & Medical Encyclopaedia, 1976).

- Religion & Life style

Majority of the subjects were Christians (67% G.A.R. and 85% U.E.R), followed by Moslems (32% G.A.R. and 5% U.E.R.) with the traditional religionists being the least (0.7% G.A.R. and 9% U.E.R.)

All subjects were non-smokers with just 0.7% being vegetarian. Alcohol users were 4% in GAR and 1.3% in UER.

- Socio-Economic Status

Close to half (47%) the mothers of subjects in the north and 21% in the south, respectively were illiterates whilst educated mothers made up 53% of the northern sample and 79% of southern sample. Thus, more of the southern respondents had educated mothers than the northern respondents. Also, only 15% of fathers of the Northern respondents earned more than ¢800,000 the rest earned less. In the south, 41% of the fathers of the study group earned over ¢800,000.

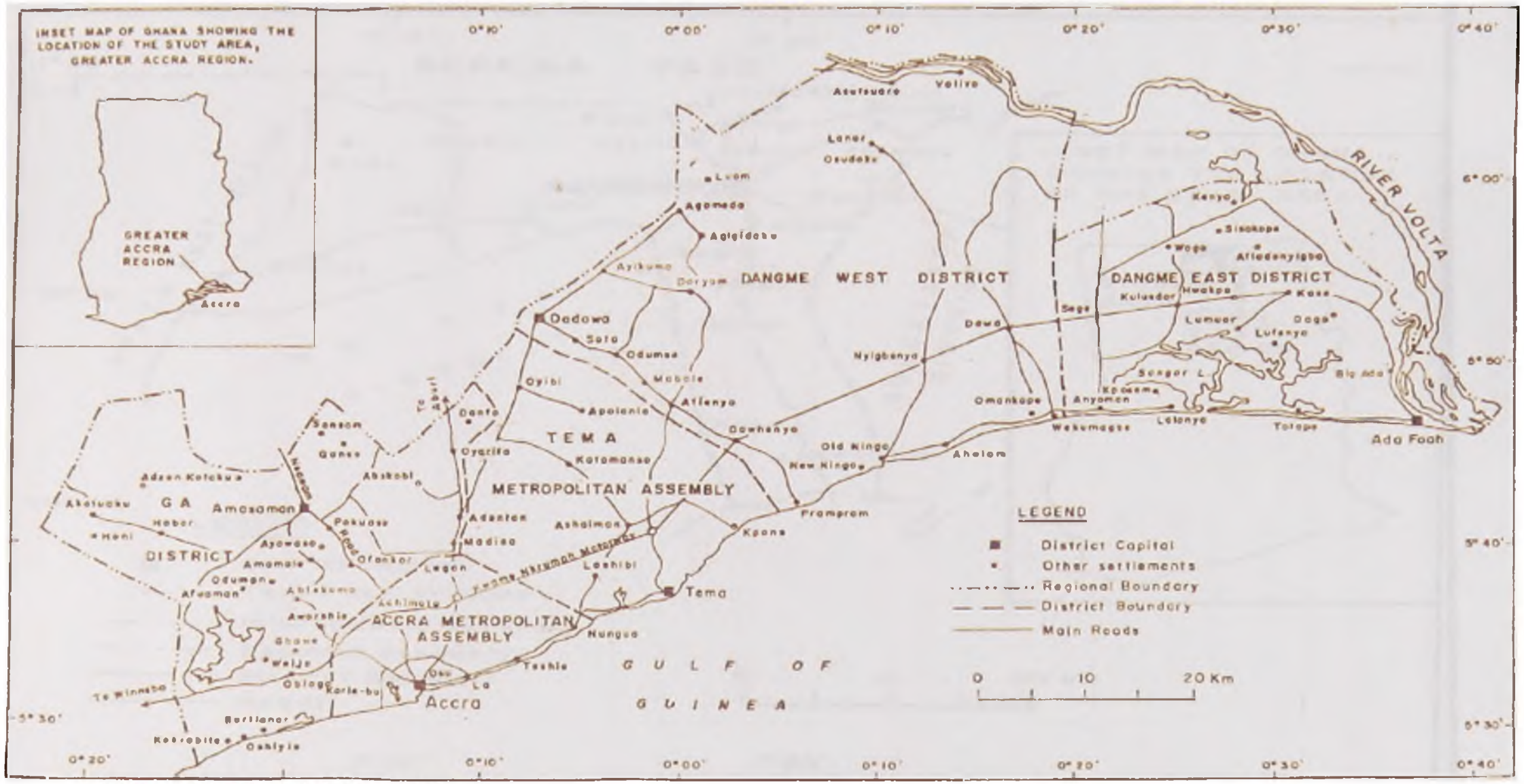
When type of accommodation was looked at, about 17% of the Southern group lived in self -contained houses with more than 2 bedrooms whilst only

1% of the northern sample could afford similar houses. A high proportion (69%) of the northern sample compared to 31% of Southern sample used untreated water. A higher percentage (33%) of the southern sample had treated water flowing through their taps everyday whereas only 5% of the northern sample could enjoy such a luxury. Charcoal (64%) and firewood (15%) are preferred fuel for cooking in the north. In the south, it is charcoal (50%) and kerosene/gas/electric stove (49%). Thus, the southern sample seemed to have a slightly higher socio-economic status than the northern sample.

- Anthropometry

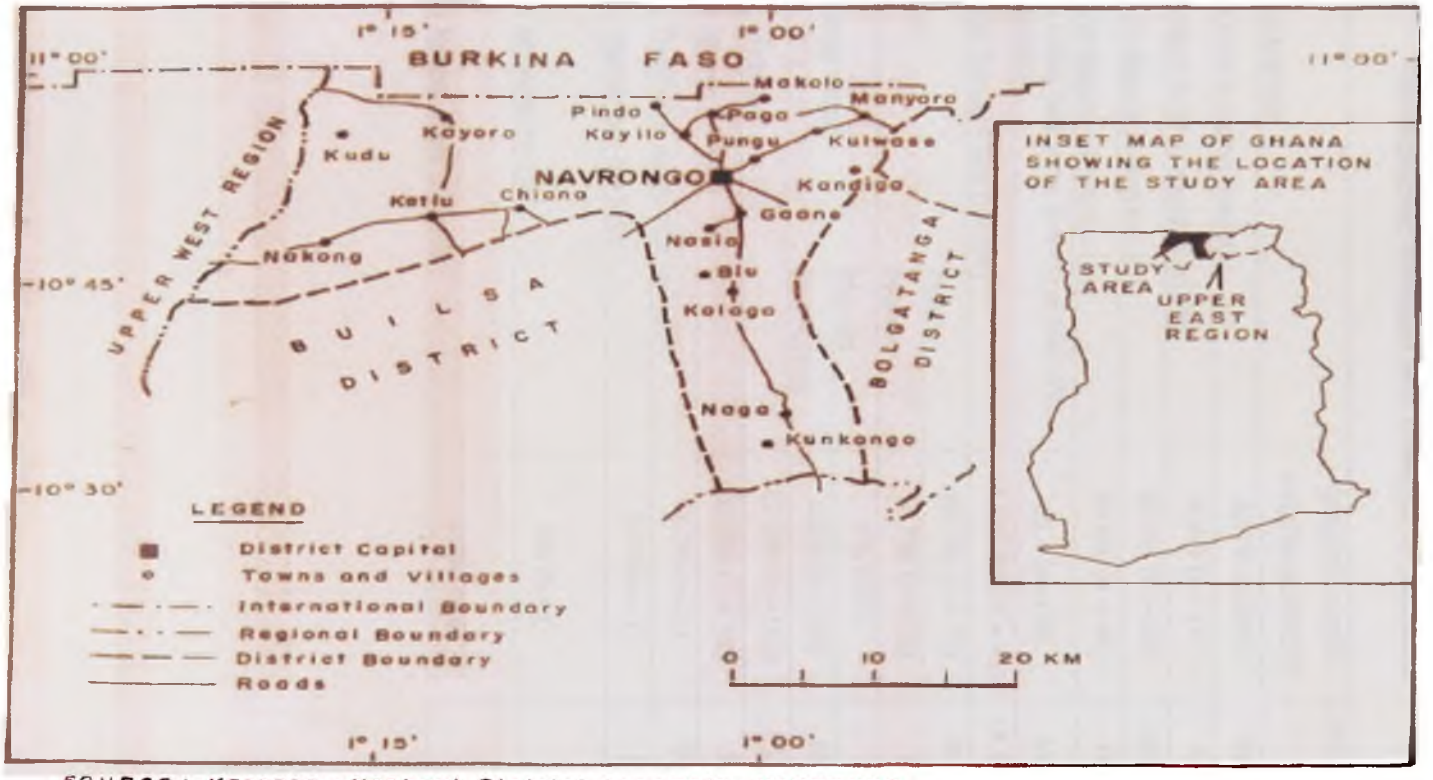
Most of the subjects from the North were underweight 63%, whilst half (51%) of the southern subjects had normal values for Body Mass Index (Table 4.3). None of the Northern sample was found to be overweight though about 3% of the southern sample was.

Fig 4.1 Map of southern study area



SOURCE ACCRA METROPOLITAN ASSEMBLY

Fig 4.2 Map of northern study area



SOURCE : Kaseena – Nankani District Assembly, NAVRONGO.

Table 4.1

Demography, Religion and Lifestyle of Subjects

CATEGORY	PARAMETER		GREATER ACCRA REGION SUBJECTS	UPPER EAST REGION SUBJECTS
Demographic Data	% Born in region (n=150)		62.7% (n=94)	89.3 (n = 134)
	% Born in other region (n=150)		9.4% (n= 14)	2.7% (n = 4)
	% in Region ≥10yrs(n=150)		69.4% (n =104)	85.3% (n=128)
	% in Region <10yrs (n=150)		30.6% (n = 46)	14.7% (n=22)
	% Living here most (>6yrs) of their life (n=150)		85.4 (n=128)	94.7% (n=142)
	Most frequent age (n=150)		14 (28.7%, n = 43)	14 (31.3%, n= 47)
	Sex	Males	49.3% (n = 74)	49.3% (n = 74)
		Female	50.7% (n = 76)	50.7(n=76)
	Marital Status	Married	0% (n=0)	0% (n=0)
		Not Married	100% (n=150)	100% (n=150)
Religion	% Christians (n=150)		67.3% (n =101)	85.3% (n =128)
	% Islamic (n=150)		32.0% (n = 48)	5.3% (n =8)
	% Traditional religionists (n=150)		0.7%(n= 1)	9.3% (n =14)
Life Style	% Vegetarians (n=150)		0.7%(n= 1)	0.7%(n= 1)
	% Smokers (n=150)		0% (n=0)	0% (n=0)
	% Alcohol users (n=150)		4.0%(n=6)	1.3%(n=2)

Table 4.2

Socio-economic status of subjects

	PARAMETER		GREATER ACCRA REGION SUBJECTS	UPPER EAST REGION SUBJECTS
Socio-economic Status	% With illiterate mothers (n=150)		21.3% (n = 32)	46.7% (n = 70)
	% With educated mothers (n=150)		78.7% (n = 118)	53.3% (n = 80)
	% Fathers -no regular income* (n=150)		4.7% (n =7)	3.3% (n =5)
	% Fathers -monthly income* < ¢800,000 (n=150)		54.7% (n =82)	81.3% (n =122)
	% Fathers -monthly income* > ¢800,000 (n=150)		40.7% (n =61)	15.4% (n =23)
	Household amenities	**Low	28.8% (n =43)	25.3% (n = 38)
		**Middle	50.7% (n =76)	58.7% (n = 88)
		**High	20.5% (n = 31)	24.0% (n = 36)
	Type of accommodation	Chamber & hall	46.0% (n = 69)	36.7% (n = 55)
		1bedroom, self contained	17.3% (n = 26)	38.0% (n = 57)
		2 bedroom, self contained	19.3% (n = 29)	24.0% (n = 36)
		> 2bedroom, self contained	17.3% (n = 26)	1.3% (n = 2)
	Water Type	No treated water	31.3% (n = 47)	68.7% (n = 103)
		Tanks/Receptacles	8.7% (n = 13)	6.0% (n = 9)
		Tap flows 2x/wk	6.7% (n = 10)	15.3% (n = 23)
		Tap flows > 2x/wk	20.0% (n = 30)	5.3% (n = 8)
		Tap flows always	33.3% (n = 50)	4.7% (n = 7)
	Cooking facility type	Firewood	0.7% (n = 1)	14.7% (n = 22)
		Charcoal	50.0% (n = 75)	64.0% (n = 96)
		Kerosene Stove	10.7% (n = 16)	7.3% (n = 11)
		Gas / Electric Cooker	38.6% (n = 58)	14.0% (n = 21)

*Subjects gave description of their Father's occupation (also confirmed from school records), and then based on that, father's income was estimated.

** A list of graded household items (see Appendix IV) was presented for selection by subjects. A score of <65 =Low SES; 65 –125 =Medium SES; >125 =High SES

Table 4.3

Summary of subjects' descriptive statistics – anthropometry

PARAMETER		GREATER ACCRA REGION SUBJECTS	UPPER EAST REGION SUBJECTS
*BMI (kg/m^2) (n=150)	Underwt (<18.50)	45.3% (n = 68)	62.7% (n = 94)
	Normal (18.50-24.99)	51.3% (n = 77)	37.3% (n = 56)
	Overwt (>24.99)	3.3% (n = 5)	0.0% (n = 0)
Ht (cm) Mean ± SD	Males	159.84 ± 10.35	154.06 ± 12.03
	Females	156.81 ± 6.19	155.69 ± 6.16
	Both Sexes	158.30 ± 8.61	154.89 ± 9.52
Wt (kg) Mean ± SD	Males	46.77 ± 9.27	41.68 ± 9.91
	Females	45.64 ± 6.93	45.64 ± 6.92
	Both Sexes	48.38 ± 9.71	43.69 ± 8.73

*BMI = Body-Mass Index =Wt (kg)/Ht^2 (metres)

❖ Health and Clinical Symptoms of Zinc Deficiency

The percentage of subjects with chronic illnesses were 9% for the south and 5% for the north with 31% Southerners and 44% Northerners having bouts of malaria and/or fever within the last one month before the study. Southern subjects who had had diarrhoea in the last one month before the study were 27%, comparable to the 28% of the northern sample. None of the southern females had ever been pregnant and only one subject in the northern sample had ever been pregnant. None was pregnant during the study. The percentage of subjects who had had health problems in the last two weeks before the study were 28% and 25% for southern and northern groups, respectively, with majority (82% and 79%) attending a health centre or hospital when sick.

About a quarter (23%) of the southern sample reported loss of appetite at the time of the study compared to just 7% from the north having the same symptom. Only 1% of northern sample had glossitis, no sign of it was observed among southern subjects.

Table 4.4

Summary of subjects' descriptive statistics on health and clinical symptoms of zinc deficiency

CATEGORY	PARAMETER	GREATER ACCRA REGION SUBJECTS	UPPER EAST REGION SUBJECTS
Health	% With chronic illnesses	8.7% (n = 13)	4.7% (n = 7)
	% With Malaria/Fever 1-4 wk before study	31.3% (n = 47)	44.0% (n = 66)
	% With diarrhoea 1-4 wk before study	26.7% (n = 40)	28.0% (n = 42)
	% Girls formerly pregnant	0.0% (n = 0)	1.3% (n = 1)
	% With health problems in the last 2wks	28.0% (n = 42)	25.3% (n = 38)
	% Attendance of health centre when sick	82.0% (n = 123)	79.3% (n = 119)
Clinical Symptoms of zinc Deficiency	Loss of appetite	22.7% (n = 34)	7.3% (n = 11)
	Glossitis	0% (n = 0)	1.3% (n = 2)

Plasma zinc and body measurements

Mean plasma zinc values recorded were 81.42 ± 26.84 for the southern sample and 82.96 ± 16.27 for the northern sample. The median, mode, minimum and maximum values are also presented on Table 4.5.

The correlation matrix (Table 4.6) revealed that Body Mass Index, age, weight, height and loss of appetite had negative associations with mean plasma zinc values. The correlation between BMI and plasma zinc was observed to have a correlation coefficient of –0.033. This means for every unit increase in BMI, plasma zinc concentration decreases by 0.033 µg/dL.

Figures 4.3 – 4.5 show plasma zinc distribution to be normal (Gaussian). This indicates that the plasma zinc values can be statistically analysed without any transformations. The closeness of the mean and median values (Table 4.5) confirms the normal distribution pattern of plasma zinc values in the population.

Table 4.5

Plasma Zinc Concentration (µg/dL) of Adolescents in Southern and Northern Ghana

Statistic	Zn Values	
	South	North
Mean ± SD	81.42 ±26.84 **(N =150)	82.96 ± 16.27 **(N=150)
Median	79.14	82.40
Mode	53.40*	80.40*
Minimum	5.16	42.10
Maximum	142.92	140.90

*Multiple modes exist. The smallest value is shown.

TABLE 4. 6: Correlation matrix among selected variables

	mean Plasma Zn (p-value)	BMI (p-value)	Age (p-value)	Weight (p-value)	Height (p-value)	Sex (p-value)	Loss of appetite (p-value)	Alcohol Intake
Mean plasma Zn	1.000							
Body Mass Index	-0.033[a] (0.574[b])	1.000						
Age	-0.020 (0.732)	0.230** (<0.001)	1.000					
Weight	-0.054 (0.353)	0.847** (<0.001)	0.350** (<0.001)	1.000				
Height	-0.043 (0.463)	0.321** (<0.001)	0.354** (<0.001)	0.769** (<0.001)	1.000			
Sex	0.014 (0.804)	0.344** (<0.001)	-0.030 (0.611)	0.188** (0.001)	-0.038 (0.513)	1.000		
Loss of appetite	-0.077 (0.183)	-0.102 (0.077)	-0.066 (0.252)	-0.055 (0.340)	0.021 (0.712)	-0.172** (0.003)	1.000	
Alcohol intake	0.096 (0.096)	-0.139* (0.016)	-0.082 (0.155)	-0.070 (0.228)	0.024 (0.674)	-0.122* (0.035)	0.046 (0.424)	1.000

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
a Correlation coefficient (r)
b p –value

Fig 4.3

Frequency Distribution of Plasma Zinc Concentrations (µg/dL) of Adolescents from Southern Ghana

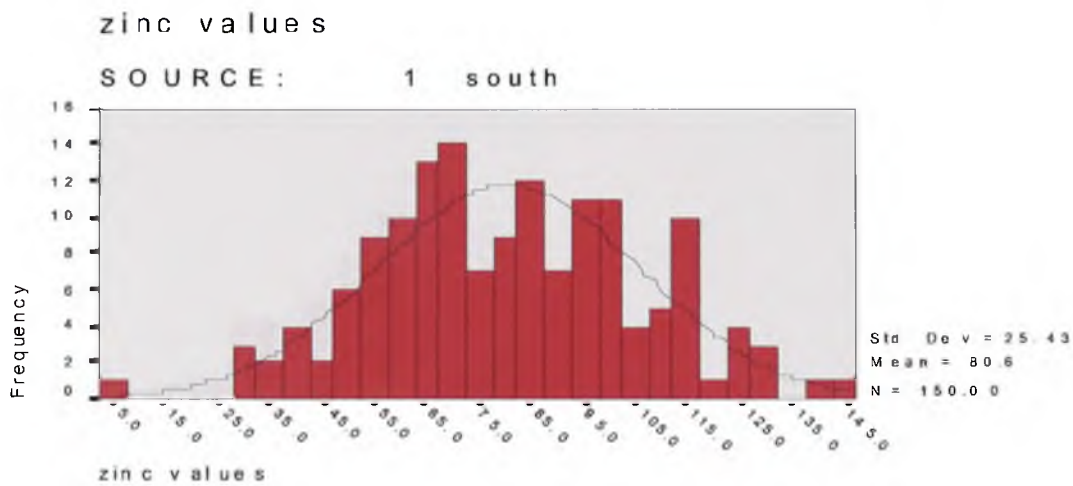


Fig 4.4

Frequency Distribution of Plasma Zinc Concentrations (µg/dL) of Adolescents from Northern Ghana

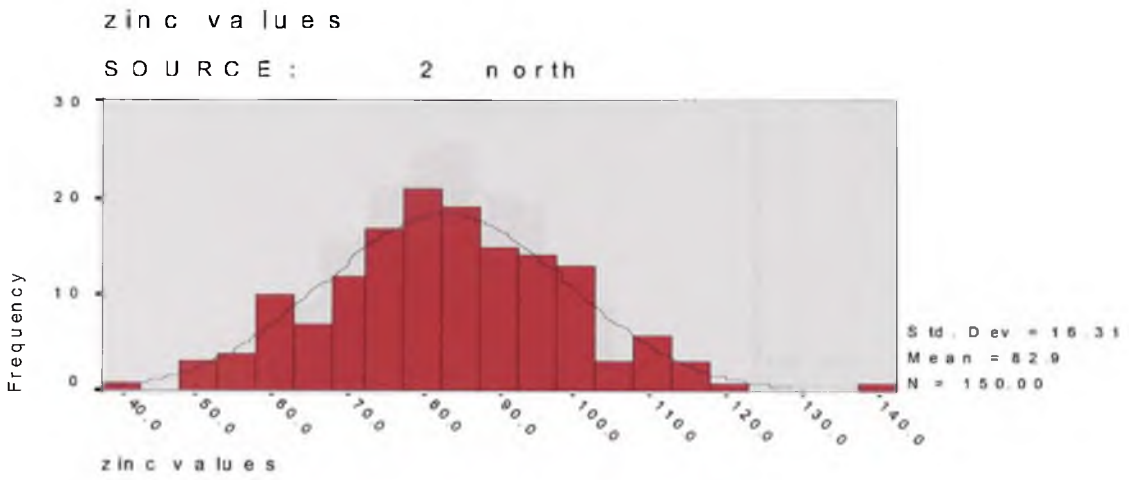
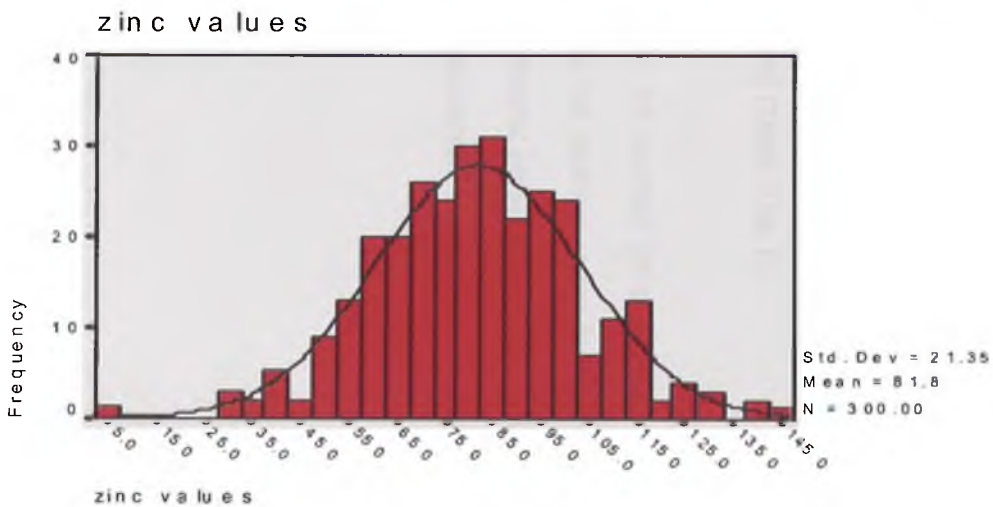


Fig 4.5

Frequency Distribution of Plasma Zinc Concentrations (µg/dL) of Adolescents in Northern and Southern Ghana (combined)



Prevalence of zinc deficiency

Table 4.7 shows that of the total of 300 subjects chosen from the Ayawaso District of Accra and Kasena Nankana District in the upper East Regions, 46.3% were found to be zinc deficient using a zinc cut off point of < 80 µg/dL (Brown and Wuehler, 2000).

Fig 4.6 depicts a higher prevalence of zinc deficiency among male adolescents as compared to their female counterparts. However this was not found to be statistically significant ($p > 0.05$) (Table. 4.8a, b).

Comparing by locale (Fig. 4.6), the southern group recorded the highest level of zinc deficiency (51%) with the southern males having the highest score (53%) followed by southern females (49%) at the zinc cut -off point of < 80 µg/dL. Of the total sample population, Northern females had the least zinc deficiency (32%).

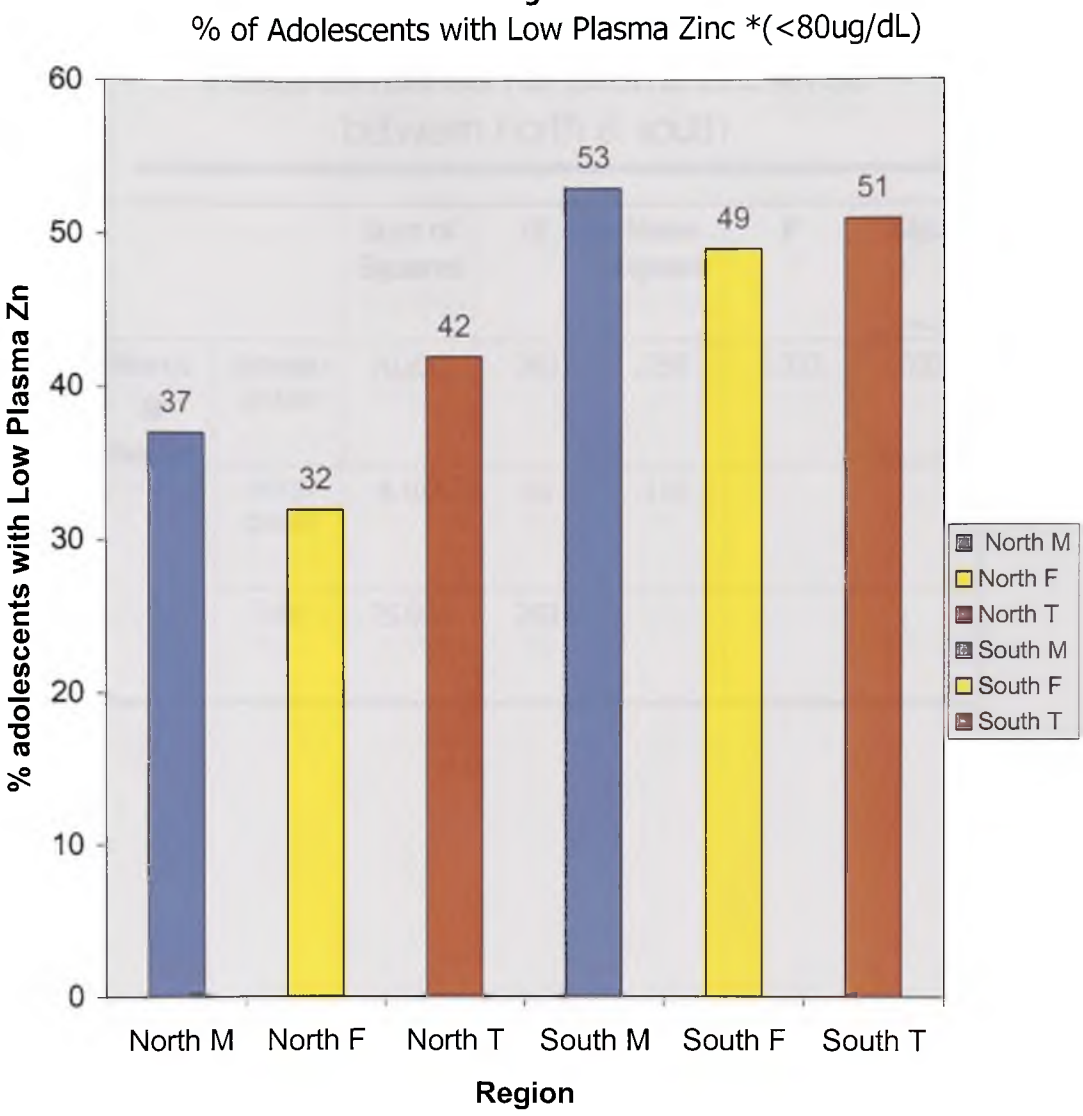
Table 4.7

Zinc deficiency among adolescents in Ghana

	NORTH	SOUTH	TOTAL
*Population zinc deficient (%)	41.5	51.1	46.3
Population with adequate stores of zinc (%)	58.1	49.2	53.7

Zinc cut off point <80µg/dL (Brown and Wuehler, 2000)

Fig 4.6:



*Reference cut of point from Brown and Wuehler, 2000.

Table 4.8a

t-tests comparison of plasma zinc levels between north & south

		Sum of Squares	df	Mean Square	F	Sig.
North & South	Between groups	70.833	263	.269	2.327	.002
	Within groups	4.167	36	.116		
	Total	75.000	299			

Table 4.8b

t-tests comparison of plasma zinc levels between males & females

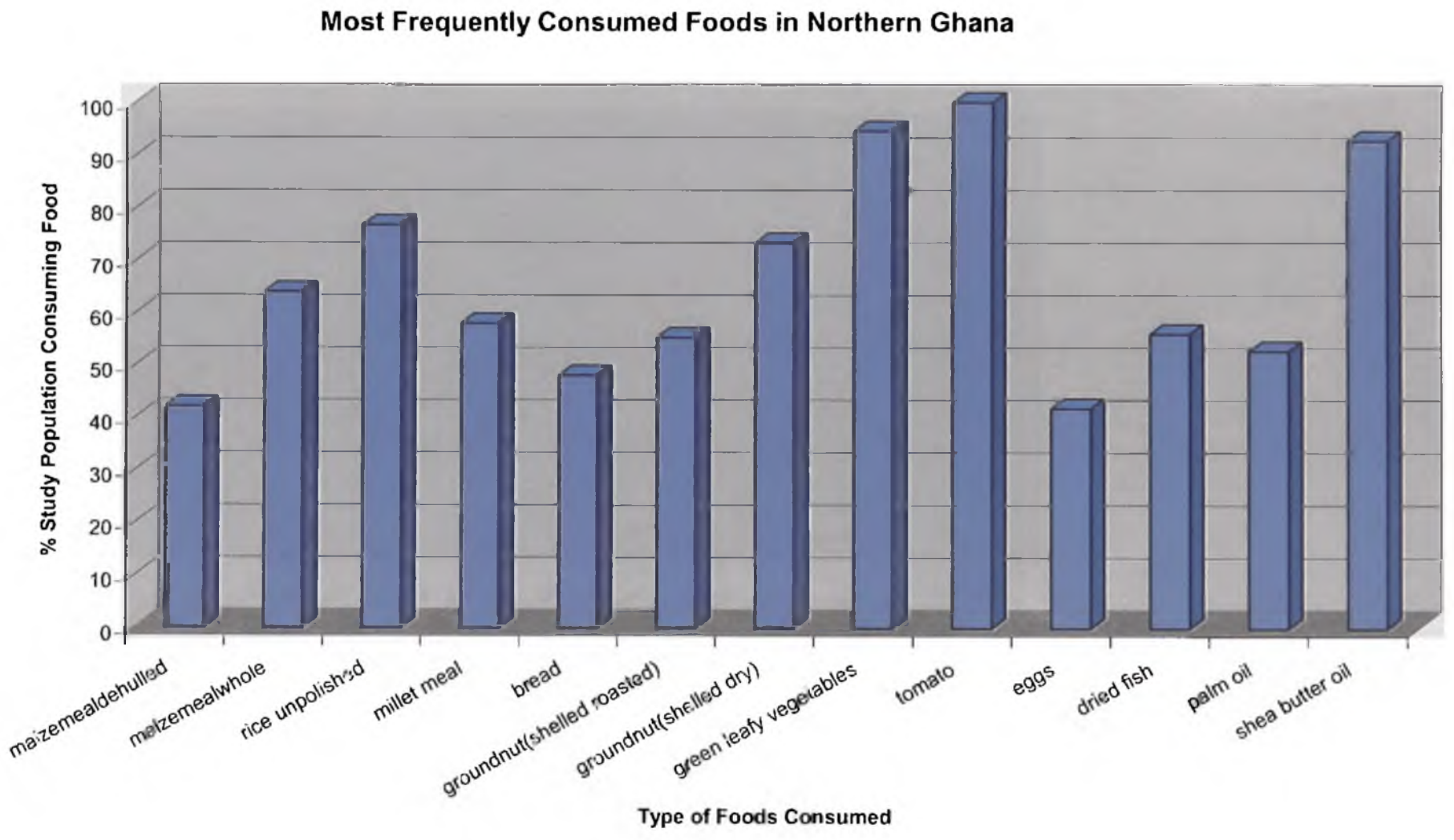
		Sum of Squares	df	Mean Square	F	Sig.
Male & Female	Between groups	66.667	263	.253	1.095	.385
	Within groups	8.333	36	.231		
	Total	75.000	299			

Frequently consumed foods

Fig 4.7 and 4.8 are graphs showing the most frequently consumed foods (i.e. foods consumed thrice or more in a week) in both study areas. A single bar means the item is most consumed in the indicated study area. Thus, some subjects in the area may consume that particular food but not as frequently as required to merit inclusion.

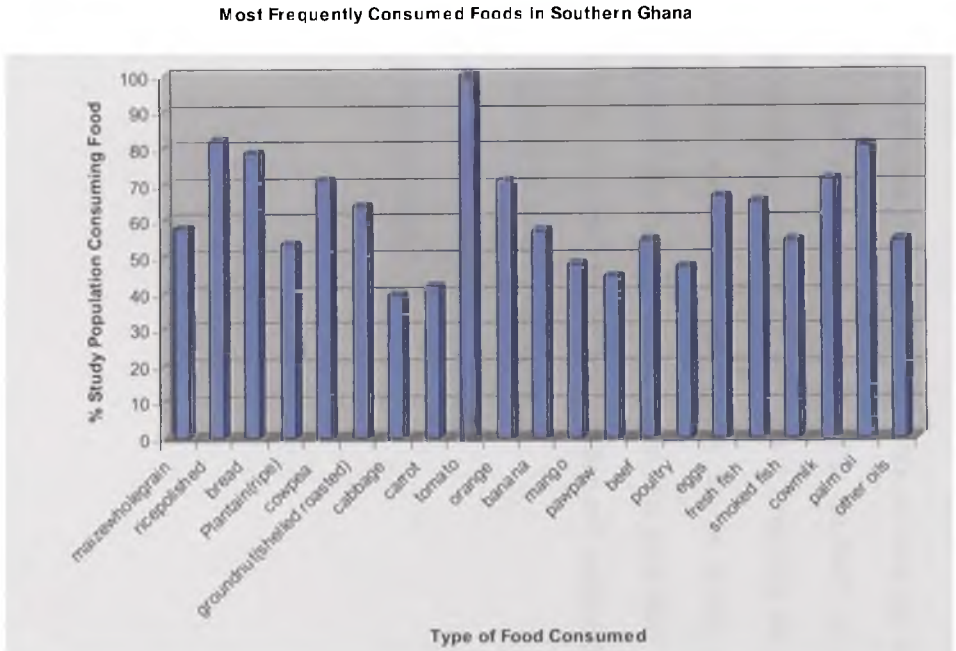
The graphs reveal that differences existed in food consumption patterns for the two study areas as expected. More cereal foods (whole and sifted maize, unpolished rice and millet) were consumed up north than in the south whilst consumption of fruits and vegetables (e.g. orange, banana, mango, pawpaw, carrot, cabbage) were more popular in the south except for tomatoes and green leafy vegetables. Of the legumes, cowpea and roasted groundnuts were the most frequently consumed in the south though up north groundnuts – both dry and roasted formed a significant part of their diet. Meat and animal products e.g. beef, poultry, eggs, milk and fish were among the most frequently consumed in the south whilst in the north, eggs (guinea fowl eggs mostly) and dried fish were among the most frequently consumed by less than 60% of the populace. Among the oils, shea butter was most prominent up north (over 90% consuming it more than thrice per week) followed by palm oil. Most frequently consumed oils in the south included palm oil and other vegetable oils such as frytol. Starchy foods such as plantain were more frequently consumed in the south as expected.

Fig 4.7



Foods eaten ≥ 3x per week per person = Most Frequently consumed foods

Fig 4.8



Foods eaten ≥ 3x per week per person = Most Frequently consumed foods

Zinc content of foods consumed

Moisture content of all most frequently consumed foods analysed are presented on Tables 4.9 and 4.10.

Figures 4.9 and 4.10 show mean (of triplicate analysis) zinc content of most frequently consumed foods (as-is basis) for both study areas. The most frequently consumed foods (i.e. Foods eaten thrice or more per week) with highest levels of zinc in the North were found to be fish* (11.2mg/100g dried) followed by shelled groundnuts (4.5 and 4.7mg/100g for dried and roasted, respectively) and millet (4.7mg/100g). In the South, the most-frequently consumed foods found to be richest in zinc were fish* (11.3 and 10.9 for smoked and fresh fish respectively), shelled and roasted groundnuts (4.8mg/100g) and beef (4.1mg/100g).

*The fish type used was Tilapia.

Moisture content of most frequently consumed foods

Table 4.9

MOISTURE CONTENT OF MOST FREQUENTLY CONSUMED NORTHERN FOODS				
FOOD ITEM	% MOISTURE		MEAN	SD
	1	2		
Millet	8.7	11.4	10.1	1.91
Dehulled Maize meal	15.7	13.8	14.8	1.34
Whole maize meal	9.5	9.6	9.6	0.07
Rice -unpolished	13.6	13.5	13.6	0.07
Bread	26.1	27.0	26.6	0.64
Groundnut, shelled, dry	9.2	10.4	9.8	0.85
Groundnut, shelled, roasted	3.0	3.2	3.1	0.14
Palm oil	1.7	1.2	1.5	0.35
Shea-butter	1.1	1.5	1.3	0.28
Green Leafy vegetables	80.1	81.2	80.7	0.78
Tomato	95.9	96.6	96.3	0.49
Fish- dry	18.0	12.4	15.2	3.96
Eggs	74.4	72.7	73.6	1.20

Table 4.10

MOISTURE CONTENT OF MOST FREQUENTLY CONSUMED SOUTHERN FOODS				
FOOD ITEM	% MOISTURE		MEAN	SD
	1	2		
Banana	76.9	77.0	77.0	0.07
Tomato	93.0	94.0	93.5	0.71
Orange	83.0	86.0	84.5	2.12
Carrot	78.0	90.0	84.0	8.49
Cabbage	92.0	90.0	91.0	1.41
Whole maize meal	11.0	10.0	10.5	0.71
Rice polished	9.0	12.0	10.5	2.12
Bread	27.7	27.4	27.6	0.21
Cowpea-dried	9.8	11.5	10.7	1.20
Groundnut -roasted	4.0	5.2	4.6	0.85
Plantain-Ripe	57.8	59.3	58.6	1.06
Mango	82.0	84.0	83.0	1.41
Pawpaw	93.0	91.8	92.4	0.85
Other oils e.g. Frytol	0.0	0.0	0.0	0.00
Beef	74.0	75.0	74.5	0.71
Poultry	73.4	73.8	73.6	0.28
Eggs	77.0	75.5	76.3	1.06
Fresh fish	78.0	80.0	79.0	1.41
Smoked fish	14.0	15.6	14.8	1.13
Cow milk, evaporated	86.0	84.0	85.0	1.41
Palm oil	0.0	0.0	0.0	0.00

Fig. 4.9

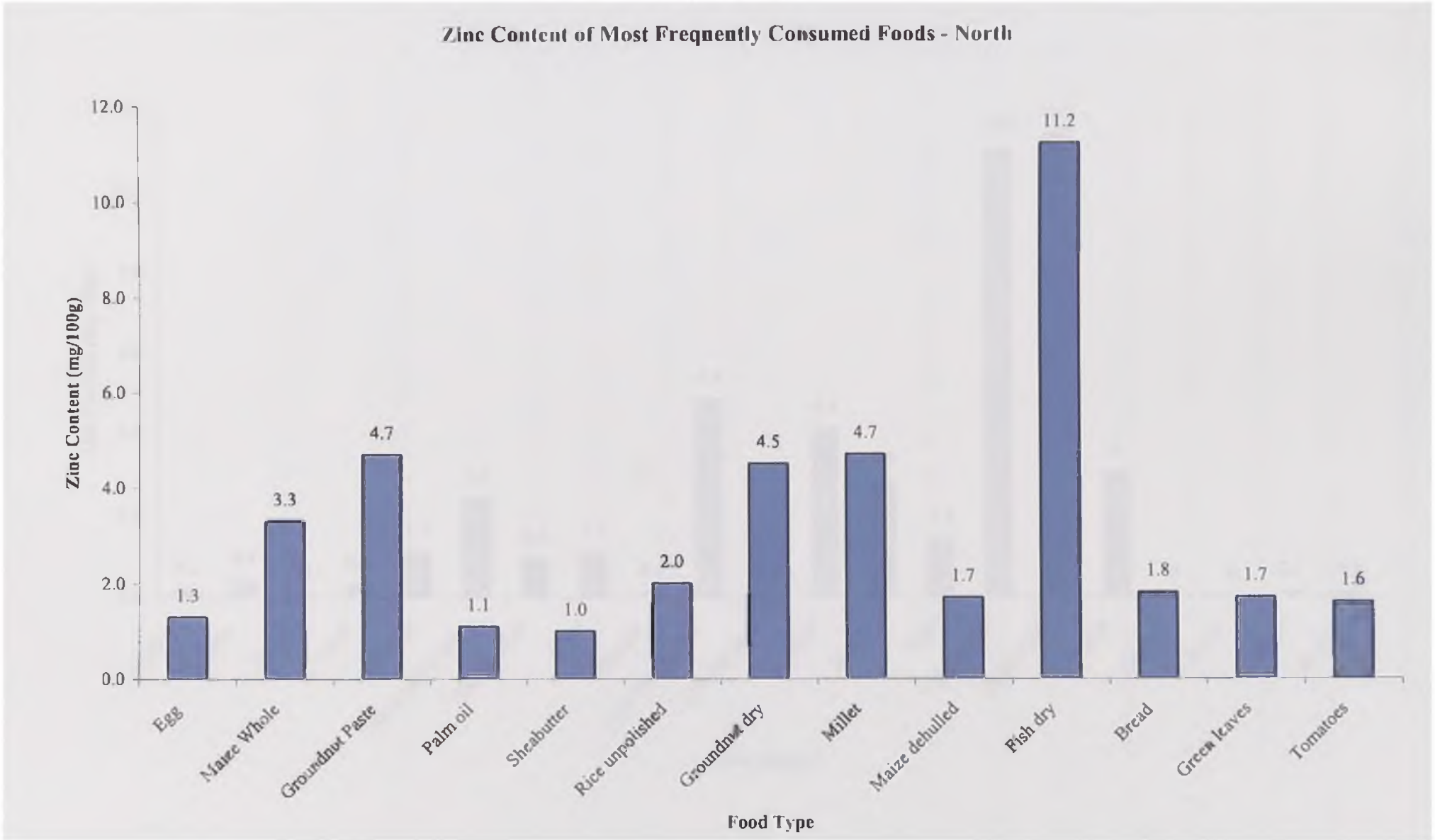
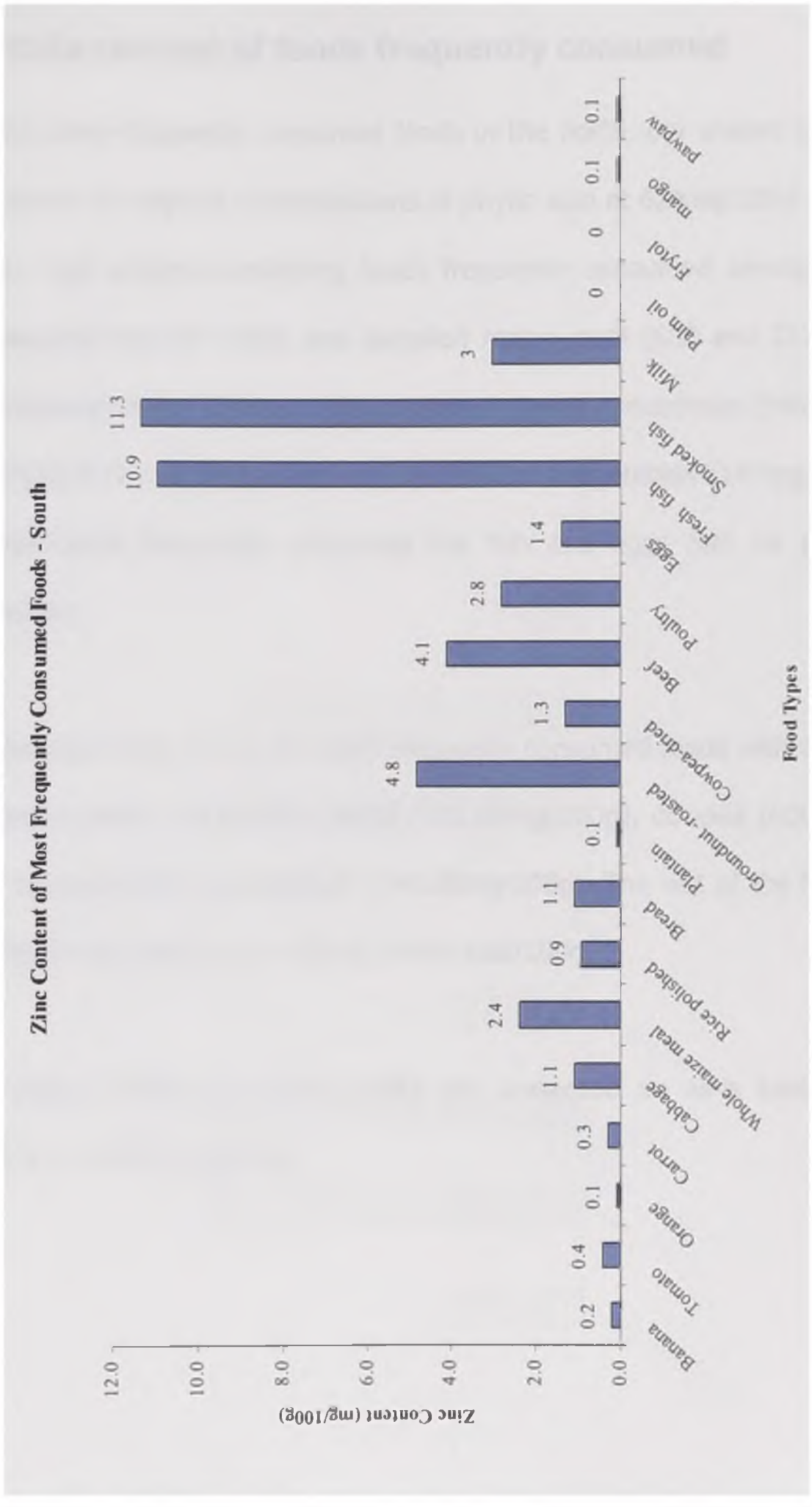


Fig.4.10



Phytate content of foods frequently consumed

Of the most-frequently consumed foods in the north, dry shelled groundnuts contained the highest concentrations of phytic acid at 623mg/100g (Fig 4.11). Other high phytate-containing foods frequently consumed among Northern adolescents include whole and dehulled maize meal (608 and 212mg/100g, respectively) millet (588mg/100g), roasted shelled groundnuts (546mg/100g), unpolished rice (220mg/100g) and green leafy vegetables (147mg/100g). All animal foods frequently consumed like fish and eggs had no phytate as expected.

In the south (Fig. 4.12), the most frequently consumed foods with the highest phytate content were whole maize (615.05mg/100g), cowpea (600mg/100g) and roasted shelled groundnuts (540.05mg/100g). The rest of the foods were found to have less than a 100mg phytic acid/100g.

All dietary phytate and zinc results are presented on as-is basis and are means of triplicate analysis.

Fig 4.11

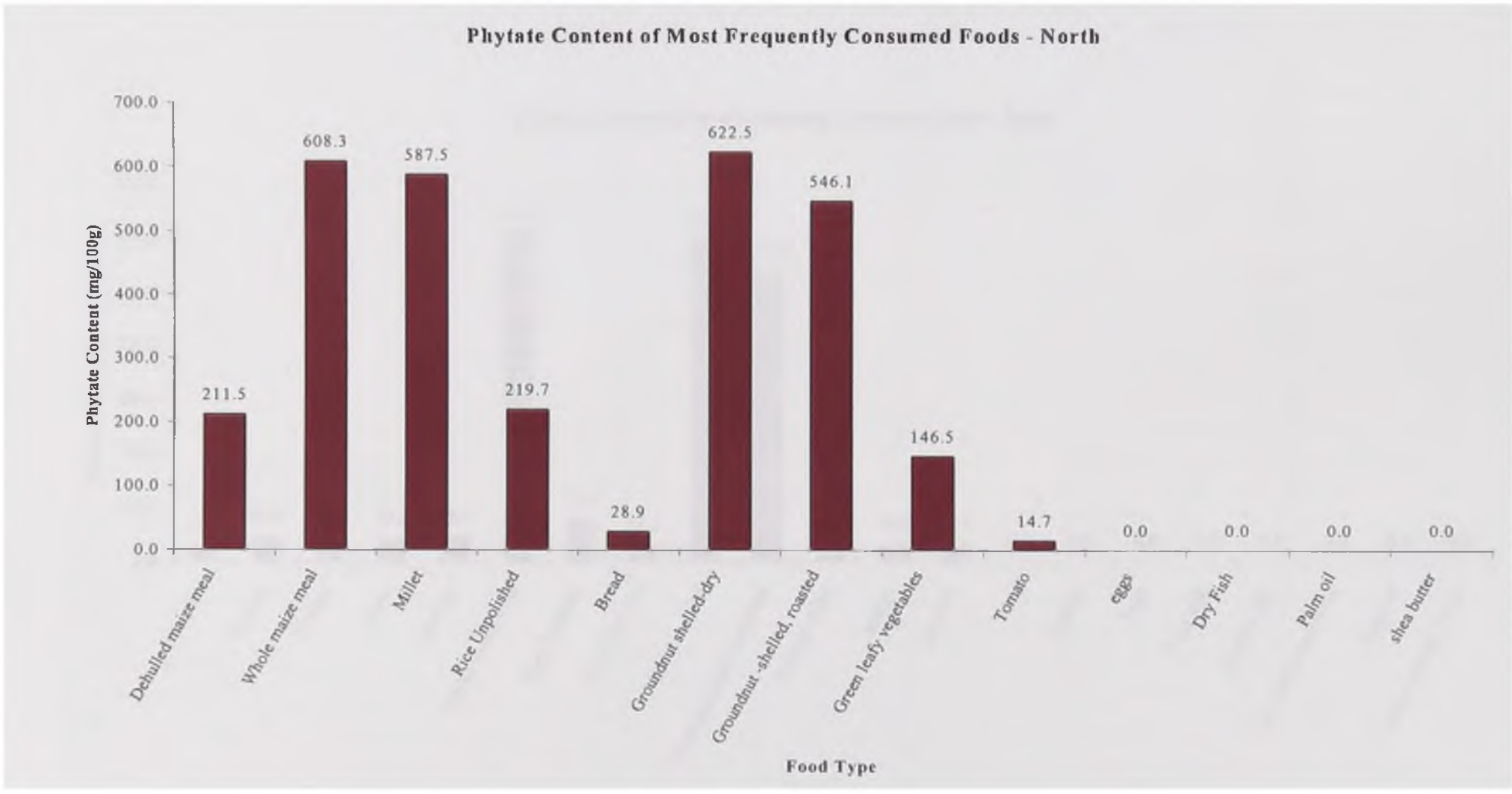


Fig 4.12

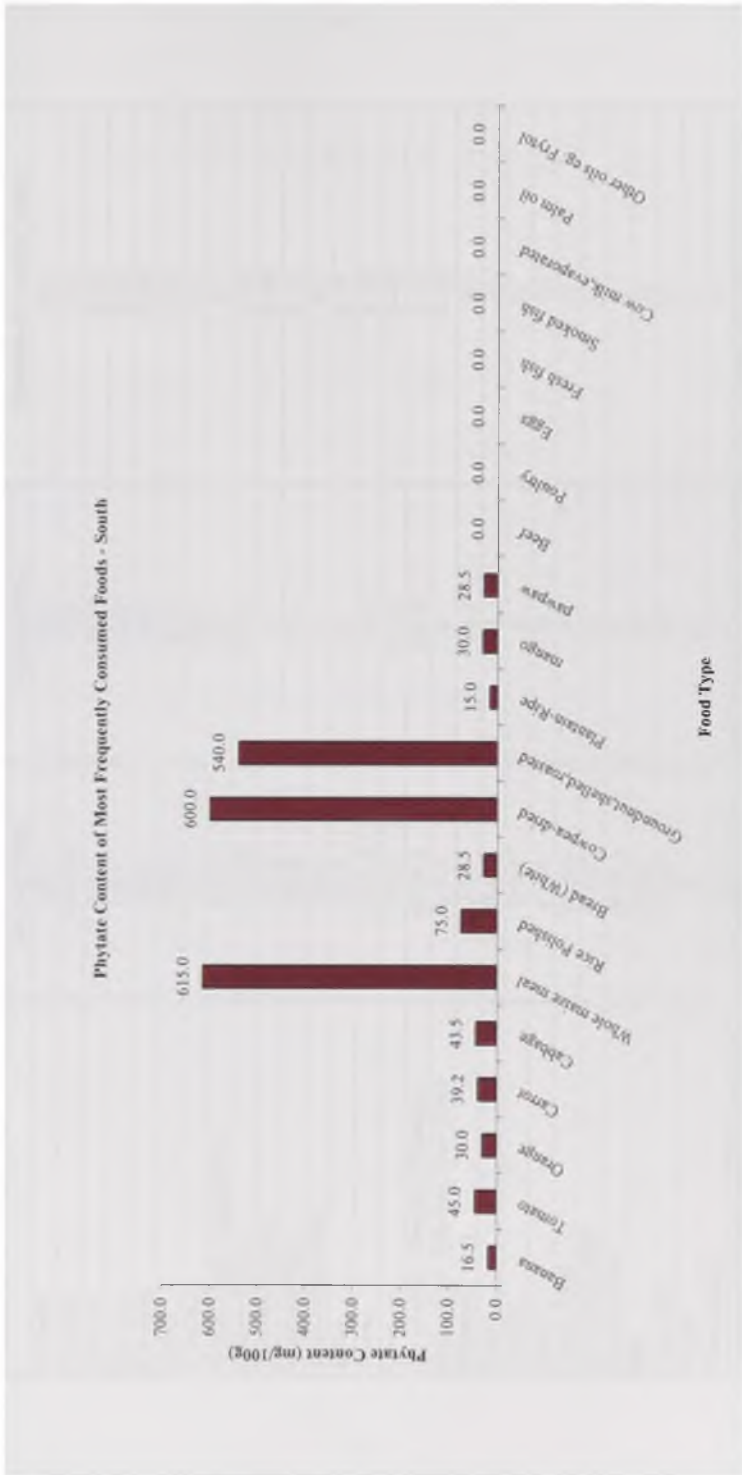


Table 4.11 Phytate- Zinc Molar Ratio of Most Frequently Consumed Foods (North + South)

Food Type	Zinc Content in mg/100g	Phytate content in mg/100g	Phytate-Zn molar ratio
Banana	0-0.2	16.5	0-8
Tomato	0.4-1.6	45.0	0-11
Orange	0-0.1	30.0	0-30
Mango	0-0.1	30.0	0-30
Pawpaw	0-0.1	28.5	0-28
Carrot	0-0.3	39.2	0-13
Cabbage	0-1.1	43.5	0-4
Green Leafy Vegetables	0-1.7	0	0
Whole Maize	2.4-3.3	615.0	9-25
Dehulled Maize	0-1.7	0	0-18
Polished Rice	0-0.9	75.0	8-12
Unpolished Rice	0-2.0	0	0
Millet	0-4.7	0	0-11
Bread	1.1-1.8	28.5	3-12
Cowpea	0-1.3	600.0	2-46
Groundnut, Shelled, Roasted	4.7-4.8	540.0	0-11
Groundnut, Shelled, sun dried	0-4.5	0	0-13
Plantain - ripe	0-0.1	15.0	15
Beef	0-4.1	0	0
Poultry	0-2.8	0	0
Eggs	0-1.4	0	0
Fresh Fish	0-10.9	0	0
Sun dried Fish	0-11.2	0	0
Smoked fish	0-11.3	0	0
Milk	0-3.0	0	0
Palm Oil	0-1.1	0	0
Frytol	0	0	0
Shea Butter	0-1.0	0	0

* Amount of zinc available for absorption estimated as 45% to 55% if phytate-zinc molar ratio < 5, as 30% to 35% if phytate zinc molar ratio = 5-15, and as 10% to 15% if phytate-zinc molar ratio > 15.

CHAPTER FIVE

DISCUSSION OF RESULTS

5.1.0 BACKGROUND DATA ON COHORTS

5.1.1 Demographic data and Religion

It was very important in this study to have subjects who had lived in the regions of study for a prolonged period of time. This is because such subjects would have assimilated the culture, eating habits and norms of the region under study. Majority of the subjects in both the Greater Accra Region and the upper East Regions were born in their respective regions and had lived there for more than half their lives. The least period of time spent by a subject in this study within their respective regions of stay was one year. It is expected that this period is long enough for acclimatisation of a subject to that study area as well as the picking up of food habits peculiar to that study site.

Religion sometimes affects dietary pattern and food habits, thus, its importance in this study. Christian subjects (67% in Accra and 85% in Navrongo) outnumbered other religions. About 32% of the Accra sample was of Islamic Religion with only 1% being Traditionalist. Among the Northern sample, only 5% belonged to the Islamic faith with 9% Traditionalists. Some

Christians and Moslems observe certain food laws such as fasting for prolonged periods during certain periods, food restrictions such as avoiding pork and alcohol (for Moslems and Jewish groups) and so on (Williams, 1993). None of the subjects within this study group was married.

5.1.2 Health History of Subjects

Information on health of subjects was collected to ascertain the presence of zinc deficiency symptoms and whether there are other confounders affecting plasma zinc levels of subjects. The most common ailments among the study group were malaria (between 31-44%) and diarrhoea (between 27 and 28%). Chronic illnesses were found in only 5-9% of the sample. An encouraging factor was that majority attended a health care centre or hospital when they fell ill. Morbidity may play a role in loss of appetite, poor food digestion and absorption and thus predispose a child to zinc deficiency. Research has however shown that with zinc supplementation some of these ailments could be reduced. For instance, randomised placebo-controlled studies in Gambia (Bates et al., 1993) and Papua New Guinea (Shankar et al, 2000) suggest that zinc may play a role in morbidity reduction related to *Plasmodium falciparum* (malaria) infections. The trial conducted in Gambia demonstrated a 32% reduction in clinic visits due to *P. falciparum* infections among those given 70 mg zinc twice weekly for 18 months. Similarly, the trail in Papua New Guinea showed a 38% reduction in clinic visits attributable to *P. falciparum*

parasitemia among pre-school children provided with 10 mg zinc daily (Shankar et al, 2000).

Only one girl from the Northern sample had ever been pregnant. Reproductive functions examined in literature in relation to zinc status are duration of pregnancy, foetal growth, the timing, sequence and efficiency of labour and delivery; and the incidence of still births and congenital malformations. Although there are clear relationships between zinc nutriture and each of these outcomes in animal models (Hurley and Baly (1982); Keen and Hurley 1989), especially when zinc deficiency is severe, the results of human studies have been less consistent possibly because of small sample sizes, other inadequacies in study design, and the difficulty in accurately classifying an individual's zinc status. There is considerable information from studies to indicate that maternal zinc nutriture can influence several aspects of reproductive function and pregnancy outcome (Caulfield et al. 1999; Goldenberg et al. 1995).

Clinical signs of zinc deficiency detected in the study population included loss of appetite (7-23%) and Glossitis (less than 2%). Research has associated zinc deficiency with reductions in appetite and may thereby contribute to deficiencies of other nutrients. The mechanisms that link zinc status to appetite control are not well understood, and it is unclear whether appetite reduction precedes growth retardation or vice versa (Shay and Mangian, 2000). Other clinical signs of zinc deficiency not researched into in this study

due to time constraints and funds but reported by William, 1993; include hypogonadism, hypogeusia and hyposmia, as well as growth retardation, alopecia, dermatitis, impaired wound healing and night blindness (Haas, 2001).

5.1.3 Lifestyle/ Socio - Economic Status

Only 1% each of northern and southern subjects was found to be vegetarian, none smoked and alcohol intake was 4% among southerners and 1% among Northerners. Vegetarian diets are known to be low in zinc content. Because animal food sources supply the major portion of dietary zinc, pure vegetarians, especially women, may be at risk of marginal zinc deficiency (Williams, 1993). Alcohol use among adolescents becomes a means of appearing more adult and assumes an increasing share of their total energy intake. Pressured by peer groups, some begin to drink at a very early age. Even mild alcohol abuse in the face of the increased nutritional requirements of adolescence compromises nutritional status (Tsui and Nordstrom, 1990).

Generally, southern subjects had a higher Socio Economic Status than northern subjects as expected. Social and economic factors are important underlying determinants of adolescent morbidity, mortality and malnutrition, including micronutrient malnutrition. In the absence of clinical, biochemical, or dietary evidence of zinc deficiency, the general level of deprivation, as assessed through socio-economic indicators, can be useful to inform on a

population's potential vulnerability to zinc deficiency (Hotz and Brown, 2004). 79% of southern subjects had a maternal educational level of at least primary education or higher compared with 53% from up North.

These trends were also observed nationally with Greater Accra having a higher maternal educational level (76.4%) of at least primary education or higher and the Upper East Region with just 17.8% (Ghana Demographic and health Survey, 2004). Maternal education has been shown to consistently be critically important to child health, nutrition and survival (Caldwell and McDonald, 1982). Lower maternal education is likely to lead to inadequate child feeding, hygiene and health seeking behaviours, which in turn are likely to be associated with increased risk of zinc deficiency among children (Engle et al., 1997; Armar- Klemesu et al. 2000).

Father's income level greater than or equal to four hundred thousand Cedis was reported for 81% of people in Accra and 61% from the north. Reliable income data are not widely available because they are difficult, time consuming and expensive to collect (Hotz and Brown, 2004). This is particularly true among populations relying on agriculture as their main means of subsistence, which constitutes a large proportion of the poor in lower-income countries, like Ghana. Similarly, collecting income data on individuals in urban populations who may have up to three different occupations, or who are self employed or working in informal or even illegal activities poses similar challenges in the assessment of income.

When accommodation and household amenities were looked at, both Accra and Navrongo sample had comparable qualities. This is probably because the Ayawaso district of Accra from which the southern subjects was taken is of a low to moderate Socio Economic Class and then also other indicators of wealth up north such as ownership of farms and cattle were also factored in. But this notwithstanding, other Socio - Economic Status indicators revealed a better status of subjects in the south as compared to those from the north. Populations with poor access to health, water and sanitation are at risk of zinc deficiency (Hotz and Brown, 2004). Southerners also used better water quality (pipe-borne) which is readily available (from taps, tanks) than Northerners (who obtained their drinking water mainly from wells, bore-hole and streams. Cooking facilities were also better for southerners (Gas/electric cookers e.t.c.) as compared to Northerners (firewood, coal pots e.t.c.).

5.1.4 Anthropometry

Mean weight of subjects was 46.0 ± 9.5kg for both sexes. Mean weight for males was 44.2 ± 9.9kg and that of their female counterparts was 47.8 ± 8.8kg. Mean weight for southern sample was 49.94 ± 9.92kg and 48.38 ± 9.71kg for the north. Thus females were just a little heavier than males and the south had heavier subjects. A higher percentage (63%) of northern subjects were underweight compared to southern subjects (45%) but 3% of southern subjects were found to be overweight.

A 1987-1988 Agro ecological nutrition survey (Alderman, 1990) based on z-score cut –off point of -2SD indicated that malnutrition was particularly high in savannah agro ecological zone, i.e. northern sections of Ghana. The rates were 36% chronic malnutrition (stunted) and 9.5% acute malnutrition (wasted). The Greater Accra Region was said to have the lowest levels with chronic and acute malnutrition being 22% and 6.5%, respectively. This study confirmed that the same trends exist today and imply that the problem of malnutrition in Ghana may be due to chronic food shortages. This is evidenced by the fact that the three northern regions, which have annual periods of drought, have consistently recorded the highest levels of stunting (Ghana Demographic and Health Survey, 2003).

It must be noted that even though growth retardation is one of the earliest manifestations of zinc deficiency (Sandstead, 1976); studies have shown that low zinc levels are not a characteristic feature of short structure per se.

5.2 PLASMA ZINC

Though several authors have suggested the likelihood of widespread zinc deficiency in low income countries (Sandstead 1991; Shrimpton 1993; Gibson, 1994), like Ghana, quantitative estimates of the percentage of the global population at risk of inadequate zinc nutriture and specific information on the prevalence of deficiency in particular settings are still lacking; in large part because the aforementioned difficulties in assessing individual zinc status.

This lack of information is a major limiting factoring in attempting to effectively discuss the results of this research.

A mean plasma zinc value of 81.75 µg/dL was measured for the entire study population at reference plasma zinc of 90 ± 10 µg/dL (Brown and Wuehler, 2000). This implies subjects having a plasma zinc concentration of 80 100µg/dL have adequate zinc levels. This study revealed a minimum plasma zinc value of 5.16µg/dl, which indicates the extent of depletion of zinc stores in some of the study subjects. A maximum plasma zinc level of 142.92µg/dL was also observed. Even though Hotz and Brown (2004), estimated 21% of the population in Ghana to be at risk of inadequate zinc intake, based on the reference zinc range, 46.3% of the 300 cohorts screened were found to be deficient in the nutrient.

A similar study by Takyi and Asibey-Berko (1999), among 200 pre-schoolers in Southern Ghana revealed no zinc deficiency at the same cut-off of 90 ± 10 µg/dL. They looked at a younger age group (<5 years of age). This could be an indication that the prevalence of zinc deficiency increases at the adolescent age when the nutritional needs are much higher. Thus, it is very important to watch the zinc nutritional needs of the adolescent. According to gender, males had a higher prevalence of zinc deficiency than females. The exact reason for this is not known, however the possible role of lifestyle might play a role.

For instance, young Ghanaian girls usually do the cooking at home and observe better hygienic practices as compared to the young adolescent boys. By locale, southerners had a higher percentage of zinc deficiency (see fig 4.6). This was not exactly what one would have expected considering that socio –economic indices and availability of animal products rich in zinc where better in the south than in the north. However, other factors could have played a role here. For example, more money is needed to pay for rent, electricity, school fees and so on in the south, thereby reducing money available for food. Dietary components of inhibitors and enhancers of zinc absorption could also have played a role.

5.3 Zinc and phytate contents of most commonly consumed foods

Generally, the diet of Northern subjects contained more of cereal-containing meals than their southern counterparts (Fig 4.7 and 4.8). The most common source of zinc comes from maize, unpolished rice as well as millet. Cereals though rich in this micronutrient are also well known for their high content of phytate and fibre, which are known to be potent inhibitors of zinc absorption (Guthrie, 1989). Thus, one would have expected the zinc status of northern subjects to be lower than those of the south. However, literature indicates the possibility of high phytate containing foods being processed to reduce their phytic acid contents through fermentation for instance (Brown and Wuehler,

2000). This when done, reduces the inhibitor factor in these foods thus making zinc more available during digestion.

But one thing this study did not look at was the preparation or the treatment given to each food before consumption. Another useful thing would have been to sample foods for analysis just before they were consumed. This would have allowed more insight into the exact quantities of zinc and phytate consumed and hence a better idea of the correlation between dietary intake of zinc and phytate and plasma zinc levels. Northerners consumed more groundnuts (both fresh and roasted) than southerners. The rich zinc content of the fish (11.2mg/100g), groundnuts (4.5-4.7mg/100g) and millet (4.7mg/100g) (Fig. 4.9 and 4.10) which were among the most frequently consumed food of the Northern sample could also have been a contributory factor to the adequate plasma zinc levels of these subjects. Fish availability in the north cannot however be said to be higher than in the south, which is closer to the sea. Even though fish is more available in the south, one must factor in its high cost and thus the quantity a family can afford.

Southerners take more of the fruits (oranges, banana, mango, pawpaw) and vegetables like carrots and cabbage which are not for the most part rich sources of zinc (Brown and Wuehler, 2000). Green leafy vegetables with a mean zinc content of 1.7mg/100g and a phytate content of 146.5mg/100g (Fig. 4.11 and 4.12) were a significant aspect of the Northern diet whereas in the South it was not frequently consumed. Though low in zinc, the high

frequency of its consumption in the north could make significant additions to the zinc intake.

Even though it is good to encourage good consumption of zinc rich food, it is necessary to note that at higher doses of zinc (160-660mg/d), anaemia and changes in immune function and lipoprotein metabolism have been observed, in addition to abnormal indices of copper status (Porter *et al*, 1977; Hooper *et al*, 1980; Chandra 1984; Patterson *et al*, 1985). Generally, zinc has low toxicity; although acute symptoms of nausea, vomiting, diarrhoea, fever, and lethargy may be observed when large doses (i.e., ≥ 1g) are consumed (Brown and Wuehler, 2000). This is because when zinc intake exceeds physiological needs by reasonably small amounts, homeostasis can be maintained by increased endogenous faecal and urinary excretion.

5.4 Bioavailability of zinc in most commonly consumed foods

Discounting the effect of zinc status, zinc absorption is determined largely by its solubility in the intestinal lumen, which in turn is affected by the chemical form of zinc and the presence of specific inhibitors and enhancers of absorption. To estimate the likely absorption of zinc from the mixed diet consumed by subjects, a phytate –zinc molar ratio was calculated (Table 4.11) as follows: (phytate content of foods/660)/(zinc content of foods/65.4),

where 660 and 65.4 represent the molecular or atomic weight of phytate and zinc, respectively.

The phytate-zinc molar ratio of most frequently consumed fruits among the study subjects: banana, orange, mango and pawpaw fall within the range reported by Brown and Wuehler, 2000 (see Table 2.2). The same similarities in phytate-zinc molar ratios were found between most frequently consumed vegetables, cereals and animal products of study samples and the values quoted by Brown and Wuehler, 2000. The vegetable and fruits had the least absorbable zinc estimates.

CHAPTER SIX

CONCLUSIONS

Based on the results of the study, the following conclusions have been arrived at:

1. Mean Plasma Zn levels of Ghanaian adolescents from Northern Ghana was 82.96 ± 16.27 µg/dL and Southern Ghana was 81.42 ± 26.83 µg /dL. Overall mean plasma zinc level was 82.01 µg /dL.

2. The study revealed a 46% prevalence of zinc deficiency among adolescents in Ghana. Comparing by locale, prevalence of zinc deficiency among adolescents from southern Ghana (51%) was found to be significantly higher than that of northern adolescents (42%) at a p level of 0.002 ($p < 0.005$).

3. Food frequency intake of subjects revealed that the most frequently consumed foods up north were maize, unpolished rice, millet, bread, groundnuts, green leafy vegetables, tomatoes, guinea fowl eggs, dried fish, palm oil and shea-butter. The most frequently consumed foods among the southerners included maize, polished rice, bread, plantain, cowpea, groundnut, cabbage, carrot, tomatoes, orange, banana,

mango, pawpaw, beef, poultry, eggs, fish, milk, palm oil and other oils such as frytol.

4. The highest zinc – containing foods among the most frequently consumed foods in the north are dry fish (11.2mg/100g), groundnuts (4.5-4.7mg/100g), millet (4.7mg/100g), whole maize (3.3mg/100g) and unpolished rice (2mg/100g) and in the south are fish (10.9-11.3mg/100g), beef (4.1mg/100g), milk (3mg/100g), poultry (2.8mg/100g) and maize (2.4mg/100g). Highest phytate containing foods among the most frequently consumed foods are groundnut (546.1 622.5mg/100g), maize (211.5 608.3mg/100g), millet (587.5mg/100g), unpolished rice (219.7mg/100g), and green leafy vegetables (146.5mg/100g) for the north and maize (615mg/100g), cowpea (600mg/100g), and groundnut (540mg/100g), for the south.

5. For both study sites, foods frequently consumed entailed high zinc containing diets as well as high phytate containing diets with estimated absorbable zinc values of less than 4mg/100g.

The high level of zinc deficiency (46%) found in this study population of adolescents indicates that zinc nutriture is an important issue, which must be given considerable attention in this country. The health of our adolescents especially when it comes to the micronutrients must be looked at again. Adolescents with a low zinc status could have growth retardation, failure of

sexual maturation, higher susceptibility to lower respiratory tract infections, resulting in morbid adults, which in turn will reduce productivity and increase poverty and mortality.

RECOMMENDATIONS

Based on the outcome of this study the following recommendations are made:

1. Since the research indicated a prevalence of Zinc deficiency of 46%, there is a need for a policy to be developed to help improve Zinc nutriture of adolescents in Ghana.
2. A bigger study on Zinc status across all age groups within the different regions of Ghana is also necessary to find out where prevalence of deficiency is highest so that such places could be targeted for intervention.
3. Food analysis of various Ghanaian foods should include zinc and phytate. Fibre, another inhibitor of dietary zinc absorption should also be looked at.
4. The preparation or treatment given to each food before consumption and the quantity consumed per person per day is necessary to help the researcher know exactly how much zinc and phytate each subject is consuming from each type of food. A 24 hour – recall or weighed food intake could give this information.

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APPENDICES

APPENDIX I: CONSENT FORMS- PARENTS

03/04/02

Dear Parent,

PERMISSION TO INCLUDE YOUR WARD IN A STUDY ON ZINC NUTRITIONAL STATUS OF ADOLESCENTS IN GHANA

Zinc is an essential nutrient required by the body for several functions including growth, healing of cuts and wounds, maintaining a sense of taste and smell and for a healthy immune system. The adolescent period, characterized by its dramatically accelerated physical, biochemical and emotional development has some of the highest nutritive needs for both males and females. However, prevalence of deficiency of an important nutrient like zinc in Ghanaian adolescents is still unknown, hence the need for this study.

This study is in fulfilment of a Master of Philosophy degree in Nutrition, at the University of Ghana, Legon. In this study, subjects of ages 13 to 19 years will be recruited from schools in two regions of Ghana: the Greater Accra and Upper East regions. Questionnaires will be administered to determine food type and food intake frequency of subjects. Subsequently a qualified

phlebotomist will collect 5ml blood samples for plasma zinc determination. The subject is free to opt out anytime he/she wants.

The Kanda 1 J.S.S. was selected as one of the sites for data collection and I wish to seek your permission to include your child in this study.

I look forward to a favourable response from you.

Yours Faithfully,

Miss Grace A. Abbey
(Student)

Prof. E. Asibey-Berko
(Supervisor)

PS: Identical letters were sent to parents of pupils of Kokomlemle 2 J.S.S., Association School, Monsignor Abatey J.S.S., St. Mary's J.S.S. and Kwarania J.S.S.

CONSENT FORM

☐ I agree for my ward to participate in this study.

☐ I do not agree for my ward to participate.

Signature _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ Date _ _/ _ _/ _ _

APPENDIX II: CONSENT FORMS –SCHOOL HEADS

Box AH 260

Achimota

Accra

03-01- 2001

The Head

Kokomlemle 2 J.S.S,

Ayawaso Sub-Metro,

Accra.

Dear Sir/Madam,

PERMISSION TO INCLUDE YOUR SCHOOL IN A STUDY ON ZINC NUTRITIONAL STATUS OF ADOLESCENTS IN GHANA

Zinc is an essential nutrient required by the body for several functions including growth, healing of cuts and wounds, maintaining a sense of taste and smell and for a healthy immune system. The adolescent period, characterized by its dramatically accelerated physical, biochemical and emotional development has some of the highest nutritive needs for both males and females. However, prevalence of deficiency of an important nutrient like zinc in Ghanaian adolescents is still unknown, hence the need for this study.

This study is in fulfilment of a Master of Philosophy degree in Nutrition, at the University of Ghana, Legon. In this study, subjects of ages 13 to 19 years will be recruited from schools in two regions of Ghana: the Greater Accra and Upper East regions. Questionnaires will be administered to determine food type and food intake frequency of subjects. Subsequently a qualified phlebotomist will collect 10ml blood samples for plasma zinc determination.

Your school was selected as one of the sites for data collection and I wish to seek your permission to recruit some of the subjects for this study from your school.

I look forward to a favourable response from you.

Yours Faithfully,

_ _ _ _ _ _ _ _ _ _ _ _

Miss Grace A. Abbey

PS: Identical letters were sent to school heads of Kanda Estate 1 J.S.S., Association School, Monsignor Abatey J.S.S., St. Mary's J.S.S. and Kwarania J.S.S

APPENDIX III:

CONSENT FORM – DISTRICT HEAD OF EDUCATION

Box AH 260

Achimota

Accra

03-01- 2001

The Head

Accra New Town Exp.,

Ayawaso Sub-Metro,

Accra.

Dear Sir/Madam,

PERMISSION TO INCLUDE SCHOOLS IN AYAWASO DISTRICT IN A STUDY ON ZINC NUTRITIONAL STATUS OF ADOLESCENTS IN GHANA

Zinc is an essential nutrient required by the body for several functions including growth, healing of cuts and wounds, maintaining a sense of taste and smell and for a healthy immune system. The adolescent period, characterized by its dramatically accelerated physical, biochemical and emotional development has some of the highest nutritive needs for both males and females. However, prevalence of deficiency of an important

nutrient like zinc in Ghanaian adolescents is still unknown, hence the need for this study.

This study is in fulfilment of a Master of Philosophy degree in Nutrition, at the University of Ghana, Legon. In this study, subjects of ages 13 to 19 years will be recruited from schools in two regions of Ghana: the Greater Accra and Upper East regions. Questionnaires will be administered to determine food type and food intake frequency of subjects. Subsequently a qualified phlebotomist will collect 10ml blood samples for plasma zinc determination.

Three schools in your district will be selected as sites for data collection and I wish to seek your permission to recruit some of the subjects for this study from your cluster of schools.

I look forward to a favourable response from you.

Yours Faithfully,

Miss Grace A. Abbey	Prof. E. Asibey-Berko
(Student)	(Supervisor)

PS: An identical letter was sent to the Head of Ghana Education Service-Kasena Nankana District, Upper East Region.

APPENDIX IV: QUESTIONNAIRE

Batch no._____ Form No._____

STUDY ON ZINC STATUS OF GHANAIAN ADOLESCENTS

QUESTIONNAIRE

1. Name of Interviewer/ code.....**NINT**

2. Date of interview ☐☐☐☐☐☐ .....**DATE**

3. District name/code..... ☐☐ **DISTRICT**

4. Name of Region/Code: _____ ☐☐ **REG**

REGION		REGIONAL CODES
Greater Accra Region	01.	**GR**
Upper East Region	02.	**UR**

A: Background / Demographic Information

1. Name of Subject_____

2. Name of School _____

3. Region of Birth (Regional Code) ☐☐ **RBIRTH**

4. Years lived in this region ☐☐ **YEARS**

5. Which region have u lived longest in? ☐☐ **ELIVED**

6. Age in years ☐☐ **AGE**

7. Birth date ☐☐☐☐☐☐ **BDATE**

8. Sex | Male | Female | **SEX**

9. Are you married? | Yes | No | **MSTATUS**

10. Which religion do you belong to? _____

11. Weight .....Kg **WT**

12. Height .....m **HT**

Health Information

13. Do you have a recurrent/chronic illness? | Yes | No | **RECILL**

If yes which illness? _____

14. When was the last time you had malaria/fever?

1. Last one week
2. Last one month **LMAL**
3. Other_____

15. When was the last time you had diarrhoea?

1. Last one week **LDIAR**
2. Last one month
3. Other_____

16. For girls only: Have you ever been pregnant? | Yes | No | **EPREG**

17. Have you had any health problems the past

2 weeks? | Yes | No | **HLTHPR**

18. Do you attend a health centre when sick? | Yes | No | **HLTHCEN**

Clinical signs of Zinc deficiency

Are the following symptoms present?

19. Loss of appetite | Yes | No | **APPET**

20. Growth retardation
(weight for height index by interviewer) | Yes | No | **GROWTH**

13. Glossitis (Swollen, reddened tongue) **GLOSS** | Yes | No

Dietary Assessment

1. Eating Habits

14. What time did you go to bed last night? | | | P.m **BED**

15. What time did you wake up this morning? a.m | | **WAKE**

16. What time did you have breakfast this morning? | | Am **BRKTM**

17. Are you a vegetarian? | Yes | No | VEGTRN

18. Do you smoke? | Yes | No | **SMOKE**

19. Do you take alcohol? | Yes | No | **ALCOHOL**

II. 24-hr Recall

Starting from the morning of yesterday, can you tell me what you ate and how much you ate?

Meal Pattern	Food Type	Amount consumed	CODING
Breakfast			BRKFAST
Lunch			LUNCH
Supper			SUPPER
Snack			SNACK

III. Food Frequency Questionnaire

For each food item, please indicate by ticking, the category that best describes the frequency with which you eat that particular food item.

Food Item	Frequency of Consumption						
	More than once/day Code 1	Once/day Code 2	3-6 times/wk Code 3	Once or 2x per wk Code 4	Once/mo Code 5	Never Code 6	Coding
CEREALS							
Maize -meal (sifted)							MMS
Maize- meal (whole)							MMW
Maize (whole grain)							MWG
Millet meal							MM
Sorghum							S
Rice (Polished)							RP
Rice (unpolished)							RU
Bread (brown)							BB
Bread (white)							BW
Others, specify							OS

STARCHY ROOTS +TUBERS							
Plantain (ripe)							PR
Plantain (unripe)							PU
Banana							B
Cassava –fufu							CF
Cassava-koko							CK
Cassava-fried							CR
Cocoyam							C
Tuo zaafi							TZ
Yam –boiled							YB
Yam- fufu							YF
Irish potato							IP
Sweet potato							SP
Others, specify							OOS

SOCIOECONOMIC INFORMATION

Subjects will be classified into one of 3 groups: Low, Middle or High SES, according to the weighted wealth index as shown below:

Mother's Education:

-No education	(5)	**MED**
-Primary school	(10)	
-Middle school/JSS	(20)	
-Secondary /SSS	(30)	
-Tertiary	(50)	

Fathers Income:

-No regular income	(0)	**FED**
-<100,000 cedis /month	(10)	
-100,000- <400,000/month	(20)	
-400,000- 800,000 cedis/month	(30)	
>800,000-1,000,000/month	(40)	
>1,000,000 Cedis/month	(60)	

Household amenities/ properties

-Iron	(5)	**HSAMEN**
-Fan	(10)	
-Black and white Television	(20)	
-Colour Television	(40)	
-Video deck	(40)	
-Refrigerator	(30)	
-Air conditioner	(50)	
-Bicycle	(20)	
-Motorcycle	(30)	

-Automobile	(60)	
-Farms	(40)	
-Cattle	(40)	

Type of Accommodation:

-Chamber and Hall	(5)	**ACCOM**
-I bedroom, self-contained	(10)	
-2 bedroom, self-contained	(20)	
->2 bedroom, self-contained	(60)	

Availability of water in house: **WATER**

-No treated water available, treated water fetched from outside home	(5)
-Tanks/receptacles available for water storage	(10)
-Tap water flows 2x in a week	(20)
-Tap flows more than 2x in a week but not always	(30)
-Tap flows always	(40)

Type of Kitchen/cooking Facilities:

KITCOOK

- Cook with firewood	(5)
- Cook with charcoal	(10)
- Cook with kerosene stove	(15)
- Cook with gas/electric cooker	(30)

APPENDIX V- ETHICAL CLEARANCE MATERIAL

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
INSTITUTIONAL REVIEW BOARD
(UNIVERSITY OF GHANA)

Phone: +(233) 21 500374 /501178
Fax: +(233) 21 502182
Email: Director@noguchi.mimcom.net
Telex No: 2556 UGL GH

P.O. Box LG581
Legon
Ghana

My Ref. No. DF.22

7th February, 2002.

Your Ref. No.

DOCUMENTARY PROOF OF ETHICAL CLEARANCE

FEDERALWIDE ASSURANCE FWA 00001824

NMIMR – IRB CPN 003/01-02

FROM	:	THE CHAIRMAN, INSTITUTIONAL REVIEW BOARD, NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
TITLE OF PROJECT	:	**PREVALENCE OF ZINC DEFICIENCY OF GHANAIAN ADOLESCENTS VERSUS THEIR DIETARY INTAKE OF ZINC AND PHYTATE**
PRINCIPAL INVESTIGATOR:		Grace Achindiba Abbey

On the 30th of January, 2002 the Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research (NMIMR) voted and approved the protocol titled "Prevalence of zinc deficiency of Ghanaian Adolescents versus their dietary intake of zinc and phytate".

SIGNATURE OF CHAIRMAN: .....
Rev. Dr. Samuel Ayete-Nyampong
(NMIMR-IRB CHAIRMAN)

CC: Professor David Ofori-Adjei MBCHB, FRCP, FWACP (Director), Noguchi Memorial Institute for Medical Research, University of Ghana, Legon.

DATE: 11TH FEBRUARY, 2002

APPENDIX VI-FOOD CONSUMPTION FREQUENCY DATA

FOOD FREQUENCY (%) INTAKE OF ACCRA SAMPLE

FOOD TYPE	FOOD FREQUENCY/ % INTAKE						
	Once/more a day	3-6x/wk	1-2x/wk	1x/mo	once in a long while	Never	Total
CEREALS							
Maize meal (sifted)	3 (2.0)	8 (5.3)	24 (16.0)	22 (14.7)	**60 (40.0)**	22.0)	150(100.0)
Maize meal (whole)	20 (13.3)	38 (25.3)	**44 (29.3)**	22 (14.7)	22 (14.7)	4 (2.7)	150(100.0)
Maize (whole grain)	34 (22.7)	**53 (35.3)**	23 (15.3	17 (11.3)	22 (14.7)	1 (0.7)	150(100.0)
Millet meal	0 (0.0)	1 (0.7)	8 (5.3)	16 (10.7)	46 (30.7)	**79 (52.7)**	150(100.0)
Sorghum	0 (0.0)	2 (1.3)	7 (4.7)	11 (7.3)	32 (21.3)	**98 (65.3)**	150(100.0)
Rice (Polished)	**63 (42.0)**	60 (40.0)	19 (12.7)	6 (4.0)	1 (0.7)	1 (0.7)	150(100.0)
Rice (unpolished)	4 (2.7)	15 (10.0)	31 (20.7)	26 (17.3)	**37 (24.7)**	**37 (24.7)**	150(100.0)
Bread (brown)	15 (10.0)	17 (11.3)	21 (14.0)	33 (22.0)	**42 (28.0)**	22 (14.7)	150(100.0)
Bread (white)	**79 (52.7)**	39 (26.0)	22 (14.7)	6 (4.0)	4 (2.7)	0 (0.0)	150(100.0)
Tuo zaafi	11 (7.3)	24 (16.0)	15 (10.0)	16 (10.7)	26 (17.3)	**58 (38.7)**	150(100.0)
Others, specify	0 (0.0)	3 (2.0)	2 (1.4)	1 (0.7)	3 (2.0)	**141(94)**	150(100.0)
STARCHY ROOTS +TUBERS							
Plantain (ripe)	**40 (26.7)**	**40 (26.7)**	29 (19.3)	22 (14.7)	19 (12.7)	12 (8.0)	150(100.0)
Plantain (unripe)	9 (6.0)	24 (16.0)	**37 (24.7)**	35 (23.3)	33 (22.0)	12 (8.0)	150(100.0)

Cassava –fufu	17 (11.3)	25 (16.7)	52 (34.7)	26 (17.3)	18 (12.0)	**93 (62.0)**	150(100.0)
Cassava-fried	0 (0.0)	2 (1.3)	6 (4.0)	17 (11.3)	32 (21.3)	**93 (62.0)**	150(100.0)
Cocoyam	2 (1.3)	18 (12.0)	29 (19.3)	37 (24.7)	**52 (34.7)**	12 (8.0)	150(100.0)
Yam –boiled	18 (12.0)	37 (24.7)	**42 (28.0)**	24 (16.0)	25 (16.7)	4 (2.7)	150(100.0)
Yam- fufu	4 (2.7)	6 (4.0)	17 (11.3)	25 (16.7)	37 (24.7)	**61 (40.7)**	150(100.0)
Irish potato	0 (0.0)	5 (3.3)	12 (8.0)	26 (17.3)	25 (16.7)	**82 (54.7)**	150(100.0)
Sweet potato	0 (0.0)	15 (10.0)	29 (19.3)	33 (22.0)	**50 (33.3)**	23 (15.3)	150(100.0)
Others, specify	0 (0.0)	0 (0.0)	1 (0.7)	1 (0.7)	5 (3.3)	**143 (95.3)**	150(100.0)
PULSES							
Bambara beans-dried	1 (0.7)	2 (1.3)	7 (4.7)	19 (12.7)	50 (33.3)	**71 (47.3)**	150(100.0)
Cowpea, whole-dried	35 (23.3)	**72 (48.0)**	28 (18.7)	9 (6.0)	4 (2.7)	2 (1.3)	150(100.0)
Kidney beans	0 (0.0)	1 (0.7)	9 (6.0)	9 (6.0)	27 (18.0)	**104 (69.3)**	150(100.0)
Soya beans	0 (0.0)	2 (1.3)	19 (12.7)	28 (18.7)	32 (21.3)	**69 (46.0)**	150(100.0)
Green beans	10 (6.7)	16 (10.7)	18 (12.0)	21 (14.0)	40 (26.7)	**45 (30.0)**	150(100.0)
Groundnut shelled/dry	11 (7.3)	15 (10.0)	23 (15.3)	29 (19.3)	35 (23.3)	**37 (24.7)**	150(100.0)
Groundnut shelled/fresh	4 (2.7)	14 (9.3)	24 (16.0)	19 (12.7)	42 (28.0)	**47 (31.3)**	150(100.0)
Groundnut shelled/roasted	44 (29.3)	**52 (34.7)**	30 (20.0)	12 (8.0)	11 (7.3)	1 (0.7)	150(100.0)
Others, specify	1 (0.7)	0 (0.0)	1 (0.7)	1 (0.7)	3 (2.0)	**143 (95.3)**	150(100.0)
SEEDS							
Cashew nuts	2 (1.3)	1 (0.7)	7 (4.7)	19 (12.7)	39 (26.0)	**82 (54.0)**	150(100.0)

Coconut	11 (7.3)	24 (16.0)	32 (21.3)	37 (24.7)	**43 (28.7)**	3 (2.0)	150(100.0)
Melon seeds/Egushi	2 (1.3)	18 (12.0)	39 (26.0)	31 (26.7)	**44 (29.3)**	16 (10.7)	150(100.0)
Others, specify	0 (0.0)	0 (0.0)	1 (0.7)	1 (0.7)	2 (1.3)	**146 (97.3)**	150(100.0)
VEGETABLES							
Cowpea leaves	0 (0.0)	1 (0.7)	4 (2.7)	7 (4.7)	11 (7.3)	**127 (84.7)**	150(100.0)
Cassava leaves	0 (0.0)	1 (0.7)	5 (3.3)	8 (5.3)	9 (6.0)	**127 (84.7)**	150(100.0)
Kapok leaves	1 (0.7)	3 (2.0)	3 (2.0)	3 (2.0)	11 (7.3)	**129 (86.0)**	150(100.0)
Cocoyam leaves	9 (6.0)	25 (16.7)	**50 (33.3)**	35 (23.3)	24 (16.0)	7 (4.7)	150(100.0)
Taro leaves	1 (0.7)	1 (0.7)	11 (7.3)	6 (4.0)	10 (6.7)	**121 (80.7)**	150(100.0)
Pumpkin leaves	0 (0.0)	3 (2.0)	2 (1.3)	3 (2.0)	16 (10.7)	**126 (84.0)**	150(100.0)
Cabbage	24 (16.0)	**35 (23.3)**	34 (22.7)	26 (17.3)	24 (16.0)	7 (4.7)	150(100.0)
Carrot	22 (14.7)	**41 (27.3)**	33 (22.0)	24 (16.0)	26 (17.3)	4 (2.7)	150(100.0)
Garden eggs	14 (19.3)	37 (24.7)	**47 (31.3)**	28 (18.7)	20 (13.3)	4 (2.7)	150(100.0)
Lettuce	7 (4.7)	15 (10.0)	18 (12.0)	**38 (25.3)**	35 (23.3)	37 (24.7)	150(100.0)
Sweet potato leaves	0 (0.0)	5 (3.3)	5 (3.3)	4 (2.7)	13 (8.7)	**123 (82.0)**	150(100.0)
Okro leaves	1 (0.7)	4 (2.7)	6 (4.0)	2 (1.3)	7 (4.7)	**130 (86.7)**	150(100.0)
Other leaves, specify	1 (0.7)	1 (0.7)	2 (1.3)	1 (0.7)	3 (2.0)	**142 (94.7)**	150(100.0)
FRUITS							
Banana	**41 (27.3)**	**45 (30.0)**	43 (28.7)	12 (8.0)	6 (4.0)	3 (2.0)	150(100.0)
Tomatoes	**142 (94.7)**	5 (3.3)	1 (0.7)	1 (0.7)	1 (0.7)	0 (0.0)	150(100.0)
Apple	3 (2.0)	7 (4.7)	32 (21.3)	45 (30.0)	**49 (32.7)**	14 (9.3)	150(100.0)
Orange	**71 (47.3)**	36 (24.0)	32 (21.3)	5 (3.3)	5 (3.3)	1 (0.7)	150(100.0)

Avocado Pear	6 (4.0)	21 (14.0)	26 (17.3)	28 (18.7)	**37 (24.7)**	32 (21.3)	150(100.0)
Lemon	0 (0.0)	2 (1.3)	0 (0.0)	6 (4.0)	64 (42.7)	**78 (52.0)**	150(100.0)
Mango	29 (19.3)	**43 (28.7)**	32 (21.3)	21 (14.0)	22 (14.7)	3 (2.0)	150(100.0)
Water melon	14 (9.3)	29 (19.3)	36 (24.0)	21 (14.0)	**41 (27.3)**	9 (6.0)	150(100.0)
Pawpaw	28 (18.7)	**39 (26.0)**	30 (20.0)	25 (16.7)	22 (14.7)	6 (4.0)	150(100.0)
Pineapple	26 (17.3)	31 (20.7)	**47 (31.3)**	23 (15.3)	20 (13.3)	3 (2.0)	150(100.0)
Guava	0 (0.0)	8 (5.3)	10 (6.7)	12 (8.0)	**71 (47.3)**	49 (32.7)	150(100.0)
Others, specify	0 (0.0)	2 (1.3)	15 (10.0)	51 (34.0)	**57 (38.0)**	25 (16.7)	150(100.0)
MEAT							
Beef	37 (24.7)	**45 (30.0)**	34 (22.7)	16 (10.7)	17 (11.3)	1 (0.7)	150(100.0)
Mutton	19 (12.7)	39 (26.0)	**44 (29.3)**	25 (16.7)	16 (10.7)	7 (4.7)	150(100.0)
Poultry	29 (19.3)	**42 (28.0)**	**42 (28.0)**	19 (12.7)	15 (10.0)	3 (2.0)	150(100.0)
Guinea fowl	0 (0.0)	1 (0.7)	1 (0.7)	20 (13.3)	45 (30.0)	**83 (55.3)**	150(100.0)
Game meat	2 (1.3)	4 (2.7)	9 (6.0)	19 (12.7)	43 (28.7)	**73 (48.7)**	150(100.0)
Eggs	**55 (36.7)**	45 (30.0)	26 (17.3)	15 (10.0)	7 (4.7)	2 (1.3)	150(100.0)
Termite	0 (0.0)	0 (0.0)	1 (0.7)	3 (2.0)	7 (4.7)	**139 (92.7)**	150(100.0)
Others, specify	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	**149 (99.3)**	150(100.0)
FISH							
Fresh fish	**62 (41.3)**	36 (24.0)	25 (16.7)	12 (8.0)	11 (7.3)	4 (2.7)	150(100.0)
Dry fish	28 (18.7)	28 (18.7)	**47 (31.3)**	20 (13.3)	19 (12.7)	8 (5.3)	150(100.0)
Smoked fish	**49 (32.7)**	33 (22.0)	39 (26.0)	14 (9.3)	15 (10.0)	0 (0.0)	150(100.0)

Salted fish	13 (8.7)	26 (17.3)	**40 (26.7)**	36 (24.0)	32 (21.3)	3 (2.0)	150(100.0)
MILK							
Cow milk, evaporated	**64 (42.7)**	43 (28.7)	26 (17.3)	9 (6.0)	7 (4.7)	1 (0.7)	150(100.0)
Sheep milk, fresh	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (4.0)	**144 (96.0)**	150(100.0)
Goat milk, fresh	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.3)	**148 (98.7)**	150(100.0)
COOKING FATS							
Palm oil	**72 (48.0)**	49 (32.7)	23 (15.3)	4 (2.7)	2 (1.3)	0 (0.0)	150(100.0)
Coconut oil	18 (12.0)	19 (12.7)	**38 (25.3)**	16 (10.7)	37 (24.7)	22 (14.7)	150(100.0)
Shea butter oil	0 (0.0)	4 (2.7)	4 (2.7)	6 (4.0)	28 (18.7)	**108 (72.0)**	150(100.0)
Others, specify	**60 (40.0)**	22 (14.7)	12 (8.0)	4 (2.7)	9 (6.0)	43 (28.7)	150(100.0)

FOOD FREQUENCY (%) INTAKE OF NAVRONGO SAMPLE

FOOD TYPE	FOOD FREQUENCY/% INTAKE						
	Once/more a day	3-6x/wk	1-2x/wk	1x/mo	once in a long while	Never	Total
CEREALS							
Maize meal (sifted)	30 (20.0)	**33 (22.0)**	30 (20.0)	21 (14.0)	32 (21.3)	4 (2.7)	150(100.0)
Maize meal (whole)	**62 (41.3)**	34 (22.7)	23 (15.3)	14 (9.3)	7 (4.7)	10 (6.7)	150(100.0)
Maize (whole grain)	23 (15.3)	24 (16.0)	29 (19.3)	32 (21.3)	**41 (27.3)**	1 (0.7)	150(100.0)
Millet meal	**57 (38.0)**	30 (20.0)	26 (17.3)	19 (12.7)	17 (11.3)	1 (0.7)	150(100.0)
Sorghum	8 (5.3)	12 (8.0)	21 (14.0)	35 (23.3)	**58 (38.7)**	16 (10.7)	150(100.0)
Rice (Polished)	10 (6.7)	15 (10.0)	25 (16.7)	**42 (28.0)**	38 (25.3)	20 (13.3)	150(100.0)
Rice (unpolished)	**67 (44.7)**	48 (32.0)	23 (15.3)	6 (4.0)	6 (4.0)	0 (0.0)	150(100.0)
Bread (brown)	1 (0.7)	2 (1.3)	9 (6.0)	13 (8.7)	35 (23.3)	**90 (60.0)**	150(100.0)
Bread (white)	30 (20.0)	**42 (28.0)**	36 (24.0)	29 (19.3)	10 (6.7)	3 (2.0)	150(100.0)
Tuo zaafi	**126 (84.0)**	17 (11.3)	2 (1.3)	2 (1.3)	3 (2.0)	0 (0.0)	150(100.0)
Others, specify	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.3)	2 (1.3)	**146 (97.3)**	150(100.0)
STARCHY ROOTS +TUBERS							
Plantain (ripe)	1 (0.7)	2 (1.3)	23 (15.3)	44 (29.3)	**67 (44.7)**	13 (8.7)	150(100.0)
Plantain (unripe)	0 (0.0)	6 (4.0)	15 (10.0)	34 (22.7)	**63 (42.0)**	32 (21.3)	150(100.0)
Cassava -fufu	1 (0.7)	4 (2.7)	20 (13.3)	39 (26.0)	**60 (40.0)**	26 (17.3)	150(100.0)
Cassava-fried	0 (0.0)	1 (0.7)	0 (0.0)	5 (3.3)	46 (30.7)	**98 (65.3)**	150(100.0)

Cocoyam	0 (0.0)	2 (1.3)	5 (3.3)	20 (13.3)	**104 (69.3)**	19 (12.7)	150(100.0)
Yam -boiled	3 (2.0)	17 (11.3)	**59 (39.3)**	51 (34.0)	19 (12.7)	1 (0.7)	150(100.0)
Yam- fufu	2 (1.3)	6 (4.0)	14 (9.3)	52 (34.7)	**55 (36.7)**	21 (14.0)	150(100.0)
Irish potato	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.3)	5 (3.3)	**143 (95.3)**	150(100.0)
Sweet potato	1 (0.7)	4 (2.7)	11 (7.3)	30 (20.0)	**102 (68.0)**	2 (1.3)	150(100.0)
Others, specify	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.7)	**148 (98.7)**	150(100.0)
PULSES							
Bambara beans-dried	11 (7.3)	34 (22.7)	**45 (30.0)**	42 (28.0)	17 (11.3)	1 (0.7)	150(100.0)
Cowpea, whole-dried	17 (11.3)	53 (35.3)	**57 (38.0)**	19 (12.7)	0 (0.0)	4 (2.7)	150(100.0)
Kidney beans	2 (1.3)	1 (0.7)	8 (5.3)	15 (10.0)	42 (28.0)	**82 (54.7)**	150(100.0)
Soya beans	4 (2.7)	1 (0.7)	10 (6.7)	24 (16.0)	**76 (50.7)**	35 (23.3)	150(100.0)
Green beans	0 (0.0)	5 (3.3)	5 (3.3)	8 (5.3)	58 (38.7)	**74 (49.3)**	150(100.0)
Groundnut, shelled, dry	**69 (46.0)**	41 (27.3)	22 (14.7)	10 (6.7)	4 (2.7)	4 (2.7)	150(100.0)
Groundnut, shelled, fresh	20 (13.3)	24 (16.0)	36 (24.0)	28 (18.7)	**41 (27.3)**	1 (0.7)	150(100.0)
Groundnut, shelled, roasted	32 (21.3)	**51 (34.0)**	40 (26.7)	19 (12.7)	8 (5.3)	0 (0.0)	150(100.0)
Others, specify	0 (0.0)	1 (0.7)	1 (0.7)	0 (0.0)	1 (0.7)	**147 (98.0)**	150(100.0)
SEEDS							
Cashew nuts	0 (0.0)	3 (2.0)	9 (6.0)	25 (16.7)	**87 (58.0)**	26 (17.3)	150(100.0)
Coconut	5 (3.3)	12 (8.0)	22 (14.7)	40 (26.7)	**59 (39.3)**	12 (8.0)	150(100.0)

Melon seeds/Egushi	1 (0.7)	1 (0.7)	10 (6.7)	30 (20.0)	**68 (45.3)**	40 (26.7)	150(100.0)
Others, specify	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**150 (100.0)**	150(100.0)
VEGETABLES							
Cowpea leaves	3 (2.0)	17 (11.3)	**48 (32.0)**	27 (18.0)	34 (22.7)	21 (14.0)	150(100.0)
Cassava leaves	0 (0.0)	0 (0.0)	1 (0.7)	2 (1.3)	19 (12.7)	**128 (85.3)**	150(100.0)
Kapok leaves	1 (0.7)	3 (2.0)	3 (2.0)	2 (1.3)	8 (5.3)	**133 (88.7)**	150(100.0)
Cocoyam leaves	0 (0.0)	1 (0.7)	6 (4.0)	17 (11.3)	**77 (51.3)**	49 (32.7)	150(100.0)
Taro leaves	0 (0.0)	1 (0.7)	3 (2.0)	2 (1.3)	4 (2.7)	**140 (93.3)**	150(100.0)
Pumpkin leaves	2 (1.3)	0 (0.0)	0 (0.0)	1 (1.3)	12 (8.0)	**135 (90.0)**	150(100.0)
Cabbage	0 (0.0)	4 (2.7)	26 (17.3)	**52 (34.7)**	**52 (34.7)**	16 (10.7)	150(100.0)
Carrot	1 (0.7)	2 (1.3)	14 (9.3)	45 (30.0)	**64 (42.7)**	24 (16.0)	150(100.0)
Garden eggs	1 (0.7)	2 (1.3)	20 (13.3)	57 (38.0)	**63 (42.0)**	7 (4.7)	150(100.0)
Lettuce	0 (0.0)	5 (3.3)	18 (12.0)	47 (31.3)	**65 (43.3)**	15 (10.0)	150(100.0)
Sweet potato leaves	0 (0.0)	0 (0.0)	1 (0.7)	2 (1.3)	15 (10.0)	**132 (88.0)**	150(100.0)
Okro leaves	0 (0.0)	0 (0.0)	0 (0.0)	7 (4.7)	12 (8.0)	**131 (87.3)**	150(100.0)
Other leaves, specify	**134 (89.3)**	8 (5.3)	0 (0.0)	1 (0.7)	0 (0.0)	7 (4.7)	150(100.0)
FRUITS							
Banana	0 (0.0)	14 (9.3)	34 (22.7)	**51 (34.0)**	46 (30.7)	5 (3.3)	150(100.0)
Tomatoes	**141 (94.0)**	9 (6.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	150(100.0)
Apple	0 (0.0)	0 (0.0)	1 (0.7)	4 (2.7)	**75 (50.0)**	70 (46.7)	150(100.0)
Orange	1 (0.7)	27 (18.0)	**45 (30.0)**	31 (20.7)	44 (29.3)	2 (1.3)	150(100.0)

Avocado Pear	0 (0.0)	1 (0.7)	8 (5.3)	10 (6.7)	**76 (50.7)**	55 (36.7)	150(100.0)
Lemon	0 (0.0)	0 (0.0)	0 (0.0)	7 (4.7)	47 (31.5)	**95 (63.8)**	150(100.0)
Mango	9 (6.0)	30 (20.0)	37 (24.7)	25 (16.7)	**47 (31.3)**	2 (1.3)	150(100.0)
Water melon	3 (2.0)	19 (12.7)	36 (24.0)	23 (15.3)	**66 (44.0)**	3 (2.0)	150(100.0)
Pawpaw	3 (2.0)	17 (11.3)	28 (18.7)	28 (18.7)	**69 (46.0)**	5 (3.3)	150(100.0)
Pineapple	2 (1.3)	12 (8.0)	24 (16.0)	26 (17.3)	**67 (44.7)**	19 (12.7)	150(100.0)
Guava	2 (1.3)	18 (12.0)	26 (17.3)	41 (27.3)	**54 (36.0)**	9 (6.0)	150(100.0)
Others, specify	0 (0.0)	2 (1.3)	15 (10.0)	51 (34.0)	**57 (38.0)**	25 (16.7)	150(100.0)
MEAT							
Beef	2 (1.3)	28 (18.7)	34 (22.7)	29 (19.3)	**47 (31.3)**	10 (6.7)	150(100.0)
Mutton	3 (2.0)	14 (9.3)	40 (26.7)	**50 (33.3)**	43 (28.7)	0 (0.0)	150(100.0)
Poultry	6 (4.0)	30 (20.0)	38 (25.3)	**44 (29.3)**	27 (18.0)	5 (3.3)	150(100.0)
Guinea fowl	3 (2.0)	28 (18.7)	34 (22.7)	37 (24.7)	**39 (26.0)**	9 (6.0)	150(100.0)
Game meat	1 (0.7)	0 (0.0)	4 (2.7)	13 (8.7)	61 (40.7)	**71 (47.3)**	150(100.0)
Eggs	16 (10.7)	**46 (30.7)**	41 (27.3)	30 (20.0)	13 (8.7)	4 (2.7)	150(100.0)
Termite	0 (0.0)	6 (4.0)	22 (14.7)	14 (9.3)	43 (28.7)	**65 (43.3)**	150(100.0)
Others, specify	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	**149 (99.3)**	150(100.0)
FISH							
Fresh fish	18 (12.0)	38 (25.3)	**47 (31.3)**	23 (15.3)	20 (13.3)	4 (2.7)	150(100.0)
Dry fish	**44 (29.3)**	40 (26.7)	28 (18.7)	17 (11.3)	18 (12.0)	3 (2.0)	150(100.0)
Smoked fish	22 (14.7)	41 (27.3)	**43 (28.7)**	27 (18.0)	17 (11.3)	0 (0.0)	150(100.0)
Salted fish	0 (0.0)	3 (2.0)	16 (10.7)	24 (16.0)	**65 (43.3)**	42 (28.0)	150(100.0)

MILK							
Cow milk, evaporated	15 (10.0)	20 (13.3)	28 (18.7)	28 (18.7)	**51 (34.0)**	8 (5.3)	150(100.0)
Sheep milk, fresh	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.3)	**148 (98.7)**	150(100.0)
Goat milk, fresh	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	**149 (99.3)**	150(100.0)
COOKING FATS							
Palm oil	16 (10.7)	**63 (42.0)**	35 (23.3)	20 (13.3)	16 (10.7)	0 (0.0)	150(100.0)
Coconut oil	4 (2.7)	3 (2.0)	9 (6.0)	19 (12.7)	51 (34.0)	**64 (42.7)**	150(100.0)
Shea butter oil	**108 (72.0)**	31 (20.7)	7 (4.7)	3 (2.0)	0 (0.0)	1 (0.7)	150(100.0)
Others, specify	0 (0.0)	5 (3.3)	4 (2.7)	13 (8.7)	53 (35.3)	**75 (50.0)**	150(100.0)

APPENDIX VII

SOME PHOTOGRAPHS ON DATA COLLECTION

a. BLOOD COLLECTION

The phlebotomist preparing a subject for blood sample collection on the field.

b. ANTHROPOMETRY

Height measurement of a subject being taken on the field.

c. QUESTIONAIRE ADMINISTRATION

Subjects filling questionnaire with a field interviewer looking on to ensure understanding and correct filling.