DETERMINATION OF SOIL PHYSICO-CHEMICAL PARAMETERS AND ASSESSMENT OF THE NUTRITIONAL STATUS OF CULTIVATED *HIBISCUSSABDARIFFA* (*L.*)AT DIFFERENT GROWTHCONDITIONS AND STAGES

BY

MUHAMMAD GETSOMUSA

DEPARTMENT OF CHEMISTRY,

AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

August, 2018

DETERMINATION OF SOIL PHYSICO-CHEMICAL PARAMETERS AND ASSESSMENT OF THE NUTRITIONAL STATUS OF CULTIVATED *HIBISCUSSABDARIFFA* (*L.*) AT DIFFERENT GROWTH CONDITIONS AND STAGES

BY

MUHAMMAD GETSOMUSA, HND (K.S.P) 2008, PGD (B.U.K) 2011
P16PSCH8075
(MSc/SCi/47766/2012-2013)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER DEGREE OF SCIENCE IN ANALYTICAL CHEMISTRY

DEPARTMENT OF CHEMISTRY,
FACULTY OF PHYSICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

August, 2018

DECLARATION

I declare that the work in this Dissertation entitled "Determination of soil physico-chemical parameters and assessment of the nutritional status of cultivated *hibiscus sabdariffa* (*l.*) at different growth conditions and stages" has been carried out by me in the Department of Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previouslypresented for another degree or diploma in this or any other institution.

_____	_____	_____
Name of student	Signature	Date

CERTIFICATION

This Dissertation entitled "DETERMINATION OF SOIL PHYSICO-CHEMICAL PARAMETERS AND ASSESSMENT OF THE NUTRITIONAL STATUS OF CULTIVATED *HIBISCUS SABDARIFFA* (*L.*) AT DIFFERENT GROWTH CONDITIONS AND STAGES" by Muhammad GetsoMUSAmeets the regulations governing the award of the degree of Masters in Analytical Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. M.S Sallau _____ _____
Chairman Supervisory Committee Signature Date
Chemistry Department,
Ahmadu Bello University, Zaria

Dr. S.E Abechi _____ _____
Member Supervisory Committee Signature Date
Chemistry Department,
Ahmadu Bello University, Zaria

Dr. S. Uba _____ _____
Member Supervisory Committee Signature Date
Chemistry Department,
Ahmadu Bello University, Zaria

Prof. A.O. Oyewale _____ _____
Head of Department Signature Date
Chemistry Department,
Ahmadu Bello University, Zaria

Prof. A. Z. Abubakar_____ _____
Dean, School of Postgraduate Studies Signature Date
Ahmadu Bello University, Zaria

DEDICATION

This thesis is dedicated to Almighty Allah and to my lovely grandmother late Asma'u Armaya'u.

ACKNOWLEDGEMENTS

With heartfelt gratitude, I wish to acknowledge my supervisors Prof. M.S. Sallau, Dr. S.E. Abechi and Dr. S. Uba for their guidance. Your contributions are highly appreciated.

A Special greeting goes to my tutors, Prof. A.I. Magashi and Dr. S. Uba for their guidance and advice in the course of this research.

My gratitude goes to the Head of Department Prof A.O. Oyewale and the entire staff of the Department of Chemistry, ABU, Zaria.

I am very grateful to my father in-laws Prof. T.S Bichi and Prof. S.S Bichi for their encouragement and prayers throughout the study period.

Similarly, I wish to thank my friends at home, Muhammad Sunusi Lawan, Hamza Shi'itu Abubakar Habibu,Habibu A. Musa, Kabiru Shuai'bu Getso, Saed Shafi'u and others for their prayers and moral support throughout this study, may Allah reward them abundantly.

Finally, my heartfelt gratitude goes to my lovely parents and family especially my wife and sons; Muhammad Auwal Muhammad, Muhammad Kamaluddeen Muhammad, Muhammad ShamsuddeenIbrahim and Muhammad Auwal M.Sunusi, my uncles, A. Ahmadu Suleman Jos, A. Musa SuleimanJos and M. Abdullahi Armaya'u Getso among others, for their relentless prayers and support all through, Thank you and God bless.

TABLE OF CONTENTS

Cover Page i
Declaration ii
Certification iv
Dedication v
Acknowledgements vi
Table of Contents vii
List of Tables x
List of Figures xi
List of Appendices xiii
List of Abbreviations xv
Abstract xvi
CHAPTER ONE 1
1.0 INTRODUCTION 1
1.1 Background of the Study 1
2.3 Anti-nutrients in *Hibiscus sabdariffa* 7
2.3.1 Oxalates 8
2.3.2 Tannins 9
1.3 Statement of the Research Problem 9
1.4 Justification 10
1.5 Aim of the Work 10
1.6 Objectives 11
1.7 Hypotheses 12
1.7.1 The null hypotheses (Ho) are; 12
1.7.2 The alternative hypotheses (Ho) are; 12
CHAPTER TWO 13
2.0 LITERATURE REVIEW 13
2.1 Vegetables 13
2.2 Minerals Nutrients 15
2.2.1 Calcium 15
2.2.2 Iron 15
2.2.3Magnesium 16
2.2.4Sodium 17

2.2.5 Potassium 17
2.2.6 Manganese 18
2.2.7 Copper 18
2.2.8Zinc 18
2.2.10 Fiber 19
CHAPTER THREE 21
3.0 MATERIALS AND METHODS 21
3.1 Materials 21
3.1.1Lists of Apparatus/Equipment 21
3.1.2 List of reagents used 21
3.1.4 Preparation of aqueous solutions 23
3.2 Methods 27
3. 1.1Description of the study area 27
3.1.2 Experimental Design 27
3.1.3 Crop establishment 27
3.2.1 Quality assurance 28
3.2.3Proximate, minerals and anti-nutrients analysis of the *Hibiscus sabdariffa* 31
3.2.4 Determination of physico-chemical parameters of the soil used 36
CHAPTER FOUR 39
4.0 RESULTS 39
4.1 Proximate Compositions of *Hibiscus sabariffa* 39
4.2 Minerals Compositions of *Hibiscus sabdariffa* 40
4.3 Physico-chemical parameters of the soil 41
4.4 Statistical Analysis 42
CHAPTER FIVE 64
5.0 DISCUSSIONS 64
5.1 Proximate Constituents at Growing Stages of *Hibiscus sabdariffa* 64
5.1.1 Moisture contents of *Hibiscus sabdariffa* 64
5.1.2 Ash contents of *Hibiscus sabdariffa* 65
5.1.3 Crude fat of *Hibiscus sabdariffa* 66
5.1.4 Crude proteins of *Hibiscus sabdariffa* 66
5.1.5 Crude fiber of *Hibiscus sabdariffa* 67
5.1.6 Carbohydrate of *Hibiscus sabdariffa* 68

5.1.7Calorific value of *Hibiscus sabdariffa* 69
5.1.8 Oxalate contents of *Hibiscus sabdariffa*..... 69
5.1.9 Tannin contents of *Hibiscus sabdariffa*..... 70
5.2 Minerals Components of *Hibiscus sabdariffa*at its Growing Stages 71
5.2.1 Sodium (Na) contents of *Hibiscus sabdariffa*..... 71
5.2.2 Potassium (K) contents of Hibiscus sabdariffa 72
5.2.3 Magnesium (Mg) contents of *Hibiscus sabdariffa*..... 73
5.2.4 Calcium (Ca) contents of *Hibiscus sabdariffa*..... 74
5.2.5Manganese (Mn) contents of *Hibiscus sabdariffa* 75
5.2.6 Iron (Fe) contents of *Hibiscus sabdariffa*..... 76
5.2.7 Zinc (Zn) content of *Hibiscus sabdariffa* 77
5.2.9 Copper (Cu) contents of *Hibiscus sabdariffa* 78
5.3 Soil Physicochemical Parameters..... 79
5.3.1 Nitrogen (N) contents of the soil..... 79
5.3.2 Phosphorus (P) contents 80
5.3.3 Potassium (K) contents..... 81
5.3.4 Soil pH..... 81
CHAPTER SIX 83
6.0 SUMMARY, CONCLUSION AND RECOMMENDATION 83
6.1 Summary..... 83
6.2 Conclusion 83
6.3 Recommendations..... 84
Refferences 85
Appendices 97

LIST OF TABLES

TablePage

4. 1: ANOVA multivariate tests for proximate and anti-nutrients compositions of *Hibiscus sabdariffa* grown at three treatments levels 100

4. 2: Tests of between-subjects effects of proximate and anti-nutrients contents of *Hibiscus sabdariffa* grownat three treatments levels 100

4. 3: Estimated marginal means of proximate compositions of *Hibiscus sabdariffa* grownat three treatments levels..... 101

4. 4: ANOVA multivariate tests for minerals components of *Hibiscus sabdariffa* grownat three treatments levels..... 102

4. 5: Tests of between-subjects effects for minerals components of *Hibiscus sabdariffa* at treatments ,,levels used 102

4. 6: Estimated marginal means of mineral compositions of *Hibiscus sabdariffa* attreatments levels used 103

4. 7: ANOVA multivariate tests of physico-chemical parameters of the soilusedto grow *Hibiscus sabdariffa.*..... 103

4. 8: Tests of between-subjects effects of physico-chemical parameters of the soilusedto grow *Hibiscus sabdariffa.*..... 104

4. 9; Estimated marginal means of physico-chemical parameters of the soil used togrow *Hibiscus sabdariffa.*..... 105

4. 10: Pearson correlations matrix for proximate, anti-nutrients and soil parameters from the growing stages of *Hibiscus sabdariffa.*..... 106

4. 11: Pearson correlations matrix for minerals, anti-nutrients and soil physico-chemical parameters obtained at the growing stages of *Hibiscus sabdariffa.* 107

LIST OF FIGURES

FigurePage

3. 1: Map of KUST Research Farm, Gaya indicating the sampling site 30

4.1: Proximate compositions of *Hibiscus sabdariffa* at vegetative stage..... 44

4.2 : Proximate compositions of *Hibiscus sabdariffa* at its flowering stage 45

4.3: Proximate compositions of *Hibiscus sabdariffa* at its maturity stage..... 46

4.4: Proximate compositions of *Hibiscus sabdariffa*`s calyx (fruit)..... 47

4.5: Mean calorific value of *Hibiscus sabdariffa* at its growing stages with calyx 48

4.6: Mean concentration of essential elements of *Hibiscus sabdariffa* at a vegetative..... 49

4.7: Mean concentration of essential elements of *Hibiscus sabdariffa* at its flowering stage from fertilizers at different levels..... 50

4.8: Mean concentration of essential elements of *Hibiscus sabdariffa* at its maturity 51

4.9: Mean concentration of essential elements of *Hibiscus sabdariffa`s* calyx (fruit) from fertilizers at different levels 52

4.10: Mean concentration for trace elements of *Hibiscus sabdariff*a at a vegetative growing stage from fertilizers at different levels..... 53

4.11: Mean concentration for trace elements *of Hibiscus sabdariffa* at its flowering stage on fertilizers at different levels 54

4.12: Mean concentration for trace elements of *Hibiscus sabdariffa* at its maturity stage from fertilizers at different levels 55

4.13: Mean concentration for trace elements of *Hibiscus sabdariffa`s* calyx (fruit) from fertilizers at different levels 56

4.14: Mean concentration of macro elements of soil sampled at a vegetative growth 57

4.15: Mean concentration of macro elements of soil sampled at a flowering stage of..... 58

4.16: Mean concentration of macro elements of soil sampled at a maturity stage of..... 59

4.17: Mean of soil pH level in water and KCl medium for a vegetative stage of *Hibiscus sabdariffa*..... 60

4.18: Mean of soil pH level in water and KCl medium for the flowering stage of *Hibiscus sabdariffa* 61

4.19: Mean pH level in water and KCl medium for a maturity stage of *Hibiscus sabdariffa*. 62

4. 20: Soil moisture contents across the stages of growth of *Hibiscus sabdariffa* 63

LIST OF APPENDICES

Appendix **Page**

I: Mean percentage and standard deviation for proximate and anti-nutrients constituents of *Hibiscus sabdariffa* at its growing stages and Calyx..... 97

II: Mean concentration (mg/kg) and standard deviation for minerals components of *Hibiscus sabdariffa* at its growing stages and Calyx. 98

III: Mean concentration (mg/Kg) of macro nutrients, percentage moisture, pH levels and standard deviation obtained at three growing stages of *Hibiscus sabdariffa.* .. 99

IV: Moisture contents for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 108

V: Ash content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages..... 108

VI: Crude fat content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 109

VII: Crude protein content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 109

VIII: Crude fiber content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 110

IX: Carbohydrate content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 110

X: Calorific value content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 111

XI; Oxalate content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages..... 111

XII; Tannin content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages..... 112

XIII: Na for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 112

XIV: K for *H. sabdarifa* leaves and calyx from the levels of fertilizer used at the growing stages 113

XV: Profile Plots of Mg content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 113

XVI: Ca for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 114

XVII: Mn for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 114

XVIII: Fe for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 115

XIX: Zn for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 115

XXI: Cu for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages 116

XXII: Soil N from the levels of fertilizer used at the growing stages 117

XXIII: Soil P from the levels of fertilizer used at the growing stages 117

XXIV: Soil K from the levels of fertilizer used at the growing stages 118

XXV: Soil pH in KCl from the levels of fertilizer used at the growing stages 118

XXVI: Soil pH in water from the levels of fertilizer used at the growing stages..... 119

XXVII: Vegetative Stage (SI) of *Hibiscus sabdariffa* 120

XXVIII: Flowering stage (SII) of *Hibiscus sabdariffa* 120

AXXIX: Maturity /Fruiting Stage (SIII)of *Hibiscus sabdariffa*..... 121

XXX: A dried Calyx (fruit) 121

LIST OF ABBREVIATIONS

AOAC: Association of Official Analytical Chemists
ASH: ash contents
CARB: carbohydrate
CAL: calyx
CALO: calorific value
CF: crude fiber
CFAT: crude fat
CP: crude protein
F75: 75 kg/hectare of mineral fertilizer N.P.K. 15-15-15
F100: 100 kg/hectare of mineral fertilizer N.P.K. 15-15-15
Ks: soil potassium
L: liter
MC: moisture contents
M: poultry manure
Ns: soil nitrogen
OX: oxalate
pHKCl: pH value in KCl
pHH_2O: pH value in water.
Ps: soil phosphorus
S1: vegetative stage
SII: flowering stage
SIII: maturity stage
TAN: tannin

ABSTRACT

The researchwasconducted to determinethe nutritional status of *Hibiscus sabdariffa* at different growthconditions and stages. Three levels of fertilizer treatments; poultry manure (M), 10 tons per hectare, mineral fertilizer (N.P.K. 15-15-15)at 75 kg (F75) and 100 kg (F100) per hectare were used, and the field wasweedthree times within the sampling periods. Field trials were conducted at Teaching and Research Farm of Kano University of Science and Technology, Wudil, Kano State.The treatments were laid out in complete randomizedblock design (CRBD) and poultry manure applied uniformly after land preparation before sowing.Thesamplingwas carried out between July to November, 2014 during the rainy season. Standard methods were used for the plant and soil samples collection, pre-treatment, digestion andanalysis.The results obtained for proximate constituents revealed that at a vegetative stage (SI); the application of M gave the highestcrude protein than the F75 and F100 with levels of 20.910±2.132, 15.322±5.568 and 18.426±0.704 %, respectively.Similarly, the levels of crude fiber recorded in the *H. sabdariffa* (L) were: 12.047±0.684, 11.47±9.462 and 11.173±0.0542 % for the M, F75 and F100 fertilizers at SI.Furthermore, oxalate content recorded in the *H. sabdariffa* (L) at SI was: 9.978±2.359, 8.455±3.364 and 8.190±0.213 %, respectively. It was observedthat the moisture content for M, F75 and F100were: 6.812±0.253, 6.989±1.053, and 6.504±0.354 %at SI with F75 having the higher value.The carbohydrateand tannin content at SI when the plant grown with these fertilizers was:32.366±5.555, 42.223±12.184 and 41.960±7.657 %, and 2.685±0.068, 2.756±0.114 and 2.736±0.454 %respectively and F75 obtained with higher value. Moreover, the levels of ash contentin the plant at SI when M, F75 and F100 usedwere: 12.411±0.600, 12.742±0.999 and 13,130±2.564 %, and thecrude fat recorded in the analyzed samples of *H. sabdariffa* (L) were: 15.455±2.428, 14.402±4.712 and 30.710±0.054.Similarly, calorific values that recorded: 267.400±6.948, 276.445±16.411 and 288.276 kcal in the

analyzed sampleat SIwere found to be higher with F100 among the three different fertilizer used. However, at flowering stage (SII), the ash content, crude protein, crude fiber and oxalate contentwere found to be higher when M fertilizer was usedto grow the plant. Nevertheless, F75 fertilizer wasfound higher in the levels ofcarbohydrate and tannin in the analyzedsample at SII. Moreover, whenF100wasused at thisstage,it gave the higher crude fat content.At the maturity stage(SIII), the highestmoisture content was recorded in the plant when M fertilizer was used, while higher carbohydrate and calorific value were obtained by F75 fertilizer atSIII.Conversely, thehighestash, crude protein, crude fiber, crude fat and tannin content were recorded when usingF100 fertilizer in the plant. On the other hand, the elements concentrationsat SI, using thethree different fertilizerswere:Na(448.903±1.55, 114.785±0.32 and 339.001±1.84mg/kg) for M, F75 and F100 respectively, then Mg(7881.915±14.64, 6158.574±2.56 and 4316.644±4.85 mg/kg), K(10563.292±1.40, 10399.679±2.31 and 6173.347±0.56 m/kg), Ca(33192.429±103.13, 13597.588±18.07 and 23600.721±25.90 mg/kg), Zn(30.689±0.02, 30.150±0.04 and 21.833±0.10 mg/kg) and then Cu(18.571±0.04, 10.428±0.01 and 13.222±0.04 mg/kg) for M, F75 and F100 respectively, in which M recorded highest at all.Correspondingly, F100 obtained higher in the levels of Mn andFe from M, F75 and F100 fertilizersused to grow the plant at SI were: 714.81±0.10,586.839±0.80,781.717±1.23 mg/kg and then 750.311±2.30, 922.508±2.49,1377.294 mg/kg, respectively. Generally, lead (Pb) wasnotdetected atSI. At SII, however,the M fertilizer retained the highest value for Na, K, Fe and Zn, and had theleast Pb concentration.At SIII of the plant growth, K, Fe and Znwerefound to be higher when the plant was grown with M fertilizer. More so, the levels of Mg, Mn and Cu where found to be peak at thisstage using F75 in growing the plant, while F100 fertilizer led to higher level of Na, Ca and Pb. Interestingly, the concentrations of the analyzed elements were top in the calyx when F100 was used.Although, statistically the difference was insignificant (at $p <$

0.05) on the elementsconcentration in the calyx when compared among the three fertilizes used in growing the plant. Moreover,during the plant growth some of the nutrient contentsincrease asthe mineralfertilizer concentration increases. However, Mg,K, crude fiberand oxalate content at SI,and also Zn at SI- SIII were foundtodecrease as the mineral fertilizer concentration increases (M>F75>F100). Furthermore,the soil pH at all the stages(SI- SIII), were found to be higher when M fertilizer was used among F75 and F100.The findings of this research indicated that*Hibiscus sabdariffa*(L) is a nutritious plantof rich dietary minerals and proximate compositions. While, thepoultry manure among the fertilizersstudied had the highestpotentialsfor bettercultivation of nutritious*Hibiscus sabdariffa* (L) except when crude fat contents is needed, in that caseF100 is recommendable.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Nutrition plays an important part in the etiology, management, and recovery of several medical conditions. Nigeria is a middle-income country,it is also home to the highest number of stunted children in the continent and ranks third globally with more than 10 million stunted children(Luchuo*et al*., 2013).Van de Poel *et al.*,(2008),reported thatabout 230 million under-five children are believed to be chronically malnourished in developing countries.More so, around 54% of deaths among children of this age group are believed to be associated with malnutrition in developing countries (FAO, 2008). In Sub-Saharan Africa this group about 41% be stunted and lost their life and the death increases daily in the region (FAO, 2008).

Malnutrition is widespread in Nigeria, especially in the rural areas (Olarinwaju *et al*., 2011). This is partly dueto inadequate food and nutrient supply. The 2003 Nigeria Demographic and Health Survey revealed that 38% of under-five children in Nigeria are stunted, 29% underweight and 9.2% wasted (Ajieroh, 2010). The 2004 Food Consumption and Nutrition Survey reported similar trends with 42% stunted, 25% underweight and 9% wasted (Ajieroh, 2010). These surveys indicated significant variation between the rural and urban areas with children from rural areas worse affected by malnutrition (Olarinwaju *et al*., 2011).Malnutrition in early childhood is associated with functional impairment in adult life as malnourished children are physically and intellectually less productive when they become adults (Smith and Haddad, 1999).

1.2 Roselle (*Hibiscus sabdariffa)*

1.2.1 Botanicaldescription

The genus *Hibiscus* (Malvaceae) includes more than 300 species of annual or perennial herbs, shrubs or trees (Wang *et al*., 2012) *Hibiscus sabdariffa*, Linn, which is a member of the Malvaceae family. The origin of *Hibiscus sabdariffa* is not fully known but it is believed to be native to India and Malaysia and to have been carried at an early date to Africa. It is widely grown in tropical and subtropical regions including Africa, South East Asia and some countries of America. Seeds are said to have been brought to the New World by African slaves. It is known by different synonyms and vernacular names such as "roselle" in the U.S, "l'oiselle" in France, "jamaica" or "flor de jamaica" in Mexico and Spain, "karkade" in Sudan and is commonly known as roselle, hibiscus, Jamaica sorrel or red sorrel (English) and in Arabic, karkadeh (Ali *et al*., 2005). Roselle *(Hibiscus sabdariffa)* is known by different names as wellasGuinea sorrel or bissap in Senegal, Roselle or sorrel in Asia (Morton, 1987; Glew *et al*., 1997; Lorenzo *et al*., 2000; McClintock and El Tahir, 2004; Babalola *et al*., 2001; Nyarko *et al*., 2006; Cisse *et al*., 2009a; Cisse *et al*., 2009b).

In Nigeria, its production is mainly in the Guinea and Sudan savannah zones, where the red calyx genotypes are prevalent and are the most important source of raw materials for beverages called *zoborodo.*Its native distribution is uncertain, some believe that is from India or Saudi Arabia (Ismail *et al*., 2008), while Murdock, (1959) showed evidence that *Hibiscus sabdariffa*was domesticated by the black populations of western Sudan (Africa) sometime before 4000 BC. Nowadays, it is widely cultivated in both tropical and subtropical regions (Morton, 1987 ; USDA, 2007) including India, Saudi Arabia, China, Malaysia, Indonesia, Philippines, Vietnam, Sudan, Egypt, Nigeria and México (Chewonarin *et al*., 1999; Dung *et al*., 1999; Ismail *et al*., 2008; Mahran *et al*., 1979; Rao, 1996; Yagoub Ael *et al*., 2004).

On the other hand,*Hibiscus sabdariffa* is believed to be native to Tropical Africa, but is widely distributed in the Tropics and Subtropics of both hemispheres, and in many areas of the West Indies, Central America and South East Asia (Ibrahim and Hussein, 2006). It is highly cultivated in the savannah zones of West Africa. Mexico, Egypt, Senegal, Tanzania, Mali and Jamaica are also important suppliers but production is mostly used domestically (FAO, 2004).

Hibiscus sabdariffa has relatively short growing periods and requires low inputs, as compared to other vegetables (Chadha *et al*., 2000). The potential of this leafy vegetable in playing a key role in fighting hunger and reducing malnutrition cannot be over emphasized (Watson and Eyzaguire, 2002). The young leaves and tender stems of Roselle are eaten raw in salads or cooked as greens alone or in combination with other vegetables or with meat or fish (McClintock and El Tahir, 2004). Traditional processing of the *Hibiscus sabdariffa* calyx has been greatly improved by the establishment of many small enterprises that use it for the production of jam, concentrates and particularly for drinks or beverages. Drinks and beverages made from the plant are very popular in Nigeria and highly consumed (Cissé *et al*., 2009a). The calyx is used for production of fruit drink in the tropics. Demand is on the increase because of nutraceutics endowment of the natural food drink (Salmah *et al.,* 2003). Roselle calyx has been reported to be rich in anthocyanin and dietary iron (Seguchi and Hayashi, 1998). Wang *et al.*, (1997) has confirmed the antioxidative attributes of anthocyanins and their aglycons against peroxyl radicals. Their use for the preparation of soups cuts across different cultures of West Africa, as they supply the body with minerals, vitamins, certain hormone precursors as well as proteins and energy. *Hibiscussabdariffa* calyx consumption in diet has been reported to protect the human body from degenerative diseases. A long-term consumption of diets rich in plant foods offered some protection against chronic diseases, especially cancer (Wallstrom *et al*., 2000).

1.2.1.1 Morphology

Hibiscus sabdariffa (L). is a short-day annual shrub and can grow to a height of 1–3 m, depending on variety. The green leaves are about 8–12 cm long and the stems, branches, leaf veins and petioles are reddish purple. Flowers are up to 12.5 cm wide, they are yellow with a rose or maroon eye, and are made up of five petals. After the flowers fall apart, the calyx which is a red cup-like structure consisting of 5 large sepals with a collar (epicalyx) of 8 to 12 slim pointed bracts around the base, begins to enlarge, becomes fleshy, crisp but juicy (3.2-5.7 cm long), and fully encloses the velvety capsule, (1.25-2 cm long), which is green when immature, 5-valved, with each valve containing 3 to 4 kidney-shaped light-brown-seeds, (3-5 mm long). The capsule turns brown and splits open when mature and dry (Morton 1987).

1.2.1.2 Ecology/cultivation

Hibiscussabdariffa is easy to grow in most well drained soils but can tolerate poor soils. It requires 4-8 months growth with night-time temperatures with a minimum of 20 °C, as well as 13 h of sunlight and a monthly rainfall ranging from 5– 10″ (130–250 mm) during the first few months to prevent premature flowering. Rain or high humidity during the harvest time and drying process can downgrade the quality of the calyces and reduce the yield. The quality of *Hibiscussabdariffa* is determined by seed stock, local growing conditions, and time of harvest, postharvest handling and mainly the drying step. Most of the time it grows as a supplement crop and it is susceptible to fungi, viral and bacterial attack and also to insects. A single plant produces about 1.5 kg of fruit, approximately 8 t/ha. Yields of leaves may be about 10 t/ha (EcoCrop, 2007 ; Plotto, 2004).

1.2.1.3 Uses of *Hibiscus sabdariffa*

i) Traditional culinary use

Fresh or dried calyces of *H. sabdariffa* are used in the preparation of herbal drinks, hot and cold beverages, fermented drinks, wine, jam, jellied confectionaries, ice cream, chocolates, flavouring agents, puddings and cakes (Bako *et al*., 2009; Ismail *et al*., 2008; Plotto, 2004; Rao, 1996). In Sudan and Nigeria, the calyces are boiled with sugar to produce a drink known as "Karkade" or "Zoborodo" (Gibbon and Pain, 1985). In Mexico this drink is called Jamaica or "agua *de Jamaica* "" or "*té de Jamaica*". In the West Indies the calyces can also be used as colouring and flavouring ingredient in rum (Ismail *et al.*, 2008).

The seeds are eaten roasted or ground in meals, while the leaves and shoots are eaten raw or cooked, or as a sour-flavoured vegetable or condiment (Wilson and Menzel, 1964). In Sudan, the leaves are eaten green or dried, cooked with onions and groundnuts, while in Malaysia the cooked leaves are eaten as vegetables (Ismail *et al*., 2008). In Africa, the seeds are roasted or ground into powder and used in meals, such as oily soups and sauces. In China and West Africa, the seeds are also used for their oil (Atta and Imaizumi, 2002). Another use for the seed is as a substitute for coffee (Morton, 1987).

ii) Use in local and traditional food and medicine

Hibiscussabdariffa has been widely used in local medicines. In India, Africa and Mexico, infusions of the leaves or calyces are traditionally used for their diuretic, cholerectic, febrifugal and hypotensive effects, decreasing the viscosity of the blood and stimulating intestinal peristalsis. It is also recommended as a hypotensive in Senegal (Morton, 1987), also emollient leaf pulp is used for treating external wounds and abscesses (Neuwinger, 2000). Leung, (1996) reported that an infusion of "Karkade" calyces is also used to help lower body temperature in Egypt and Sudan. It is also prepare and used in North Africa to treat sore

throats and coughs, and genital problems (Neuwinger, 2000). In India, a decoction from the seeds is used to relieve pain in urination and indigestion. However, Morton, (1987) it is used to treat liver disorders and high blood pressure in Chinese folk medicine. In Nigeria the decoction of the seeds is traditionally used to enhance or induce lactation in cases of poor milk production, poor letdown and maternal mortality (Gaya *et al*., 2009).

iii) Industrial uses

Roselle is also cultivated for the bast fibre obtained from the stems, which has many uses including weaving jute sacks, ropes, handbags and door mats (FAO, 2007). In Malaysia the oil is used to produce scrubs and soaps (Ismail *et al.*, 2008).

iv) Animal feed

The leaves are used for animal fodder and fibre (Plotto, 2004). The seeds can be used to feed poultry as well as sheep and the residue from the seeds oil extraction can also be used to feed cattle and chicks (Elamin *et al*., 2012; Morton, 1987; Mukhtar, 2007).

v) Special application of *H. sabdariffa*

Besides its importance as a food or traditional medicine in the countries of its geographic origin, hibiscus flower is traded and used worldwide today as an important ingredient in industrially produced teas and beverages. The United States and Germany are the primary markets for dried calyx. England satisfies most of its consumer's demands by importing herbal teas form Germany (Plotto, 2004). Statistics for the volume and value of dried hibiscus imported into these markets were not available, but the major clients for hibiscus importers are herbal teas manufactures, as this plant is used as base in many

herbal/fruit teas, along with apple peel, orange peel and lemon twist (McCaleb, 2000 ; Plotto, 2004).

d) Nutritional value of *Hibiscus sabdariffa (L.)*

The leaves contain protein (3.3 g/100 g), fat (0.3 g/100 g), carbohydrate (9.2 g/100 g), minerals (phosphorus (214 mg/100 g), iron (4.8 mg/100 g) thiamine (0.45 mg/100 g), ßcarotene (4135 µg/100 g), riboflavin (0.45 mg/100 g) and ascorbic acid (54 mg/100 g) (Ismail *et al*., 2008).

The nutritional composition of fresh calyx varies between studies, probably due to different varieties, genetic, environmental, ecology and harvest conditions of the plant. Early studies reported that *cHs* contains protein (1.9 g/100 g), fat (0.1 g/100 g), carbohydrates (12.3 g/100 g) and fibre (2.3 g/100 g). They are rich in vitamin C (14 mg/100 g), ß-carotene (300 µg/100 g), and calcium (1.72 mg/100 g) and iron (57 mg/100 g) (Ismail *et al.,* 2008). Mohamed et al., 2009) reported proximate the constituents of red calyx as, moisture (11.00%), ash(10.60%), crude fat(0.16%), protein(7.88%) fiber(13.20%) and carbohydrate (57.16%),Ca(60mg/100g) and Fe(25 mg/100g).The seeds contained crude fatty oil (21.85%), crude protein (27.78%), carbohydrate (21.25%), crude fibre (16.44%) and ash (6.2%). In terms of minerals, the most prevalent is potassium (1329 ± 1.47 mg/100 g), followed by sodium (659 ± 1.58 mg/100 g), calcium (647 ± 1.21 mg/100 g), phosphorus (510 ± 1.58 mg/100 g) and magnesium (442.8 ± 1.80 mg/100 g). The major saturated fatty acids identified in the seed oil are palmitic (20.84%) and stearic (5.88%) acids and the main unsaturated fatty acids are linoleic (39.31%) and oleic acid (32.06%) (Nzikou *et al*.,2011).

2.3 Anti-nutrients in *Hibiscus sabdariffa*

Vegetables contain anti-nutrient that can affect the availability of nutrients to the human body. These anti-nutrients interfere with metabolic processes and reduce the

bioavailability of nutrients from plants or plant products used as human foods (Abara, 2003). Plants generally contain chemical compounds (such as saponins, tannins, oxalates, phytates, trypsin inhibitors and nitrates) which are known as secondary metabolites and are biologically active. Some of the reported anti-nutritional factors in some plants like amaranth are phenolics, saponins, tannins, phytic acid, oxalates, protease inhibitors, nitrates and poly-phenols (Soetan and Oyewole, 2010).

2.3.1 Oxalates

Excessive amounts of oxalic acid may reduce the availability of certain minerals in the body, most notably calcium. This could be a concern especially if calcium intake levels are low to begin with, or if foods high in oxalic acid are consumed on a regular basis over long periods of time. These oxalates are known to form insoluble calcium oxalate with calcium thereby preventing the absorption and utilization of calcium by the body hence causing diseases such as rickets and osteomalacia. Accumulation of this insoluble compound in the renal glomeruli leads to the formation of renal calculi and kidney damages (Nwachukwu and Obi, 2007).

Oxalate combine with calcium to form calcium oxalate in the lumen; making calcium unavailable for absorption. The calcium oxalate is later excreted in feaces. It has been estimated that about 2-5% of the total oxalates administered is absorbed in the body; while its absorption is higher at lower doses. Free or soluble oxalate is absorbed by passive diffusion in the colon in humans (Hughes *et al*., 1992). Oxalates inhibit calcium absorption by binding it to form calcium oxalate. Oxalates also cause mineral imbalances in the body. Intake of oxalates plus fiber causes negative balance of calcium, magnesium, zinc, and copper (Kelsay *et al.,* 1979).

2.3.2 Tannins

Tannins are condensed bitter plant polyphenolic compounds which are present in high amounts in seed coats of most legumes, and certain fruits and vegetables. Tannins may precipitate proteins from aqueous solution by inhibiting digestive enzymes and have been found to interfere with digestion by displaying anti-trypsin and anti-amylase activity. Tannins chelate iron and zinc irreversibly and interfere with their absorption. The most abundant polyphenols are the condensed tannins, found in virtually all families of plants, and compromising up to 50% of the dry weight of leaves. Tannins have traditionally been considered anti-nutritional but it is now known that their beneficial or anti-nutritional characteristics depend on their chemical structure and dosage. Condensed tannins can inhibit digestion by binding to plant proteins, making them more difficult to digest. They also interfere with protein absorption and digestive enzymes activity. Other studies show that tannin, that also known as proanthocyanidins, possess various properties such as antioxidants, anti-aging, anti-apoptotic, anti-inflammatory, anti-carcinogenic as well as anti-atherosclerosis and cardiovascular protection (Soetan *et al.*, 2010).

1.3 Statement of the Research Problem

Malnutrition has been a major problem in developing countries for a long time, Arnell, (2004) reported that, by the 2090s, climate change is likely to be affecting yield in farming widen the area affected by drought, double the frequency of extreme droughts and increase their average duration six-fold. *Hibiscus sabdariffa* known as Roselle is one of the plant that can be grown on a wide range of soil conditions (Adanlawo and Ajibade, 2006), thathas relatively short growing periods and requires low inputs, compared to other vegetables (Chadha *et al.*, 2000).Thereis need for research willaddressthe issue of nutrient compositions at the growing stages of this plant whengrown with cheap fertilizer as the literature found scanty, that will helpfarmers, consumers and nutritionist preferable

patronized cultivation and utilization of the plant for nutrient supplementationas *Hibiscus sabdariffa* has relatively short growing periods and requires low inputs, compared to other vegetables (Chadha *et al*., 2000).This will be the vital information against future possible climatic changes. The research will also make authoritative contribution to literature by assessing the nutritive value of the plant when different fertilizer applied at different stages of growth and the qualitative calyx yield for commercial purposes.

1.4 Justification

It has been established that,calyx is used for production of fruit drink in the tropics. Demand is on the increase because of nutraceutics endowment of the natural food drink (Salmah *et al.,* 2003). Roselle calyx has been reported to be rich in anthocyanin and dietary iron (Seguchi and Hayashi, 1998).

Hibiscus sabdariffa is an edible plant consumes throughout its growing period in northern Nigeria. It is eaten green and fresh or dried and stored for future use. It's mostly use for organic soft drink production globally (McClintock and El Tahir, 2004). It requires low cost and labor for production as compared to other predominant crops produce. It tolerates high temperatures (30^{o}C-35^{o}C), little mineral fertilizer and resist drought. Clearly, there is a gap in knowledge related to the nature of fertilizer application in its growing and nutritional status at its growing stages and the level of anti-nutrients. *Therefore, an empirical data that will form the basisof its reliablecultivationat low cost (organically) forrecommendation to consumersforms the baseline of this research.*

1.5 Aim of the Work

The aim of this research was to determine the soil physicochemical parameters and assess the nutritional status of cultivated *Hibiscus sabdariffa*(L) at different growthconditions and stages.

1.6 Objectives

The objectivesof this research areas follows;

i. To determine the proximate constituents, essentials, and non-essential minerals content of the *Hibiscus sabdariffa* at its vegetative, flowering and maturity stages of growth.

ii. To determinethe oxalates and tannins (anti-nutrients) content at different growth conditionsand stages.

iii. To determine the soil Physicochemical parameters at different conditions and stages

1.7 Hypotheses

1.7.1 The null hypotheses (Ho) are;

a. There is no significance difference in the nutrients constituents (proximate, essential and non-essential elements) for *Hibiscus sabdariffa* at different growing stages.

b. There is no significance difference in the nutrients constituents (proximate, essential and non-essential elements) for *Hibiscus sabdariffa*among the fertilizers study.

c. There is no significance difference in the anti-nutrients content (oxalates and tannins) of the *Hibiscus sabdariffa* at its growing stages.

d. There is no significance difference in the anti-nutrients content (oxalates and tannins) of the *Hibiscus sabdariffa*among the fertilizers study.

1.7.2 The alternative hypotheses (Ho) are;

a. There is significance difference in the nutrients constituents (proximate, essential and non-essential elements) for *Hibiscus sabdariffa* at different growing stages.
b. There is significance difference in the nutrients constituents (proximate, essential and non-essential elements) for *Hibiscus sabdariffa* with different fertilizer levels.
c. There is significance difference in the anti-nutrients content (oxalates and tannins) of the *Hibiscus sabdariffa* at its growing stages.
d. There is significance difference in the anti-nutrients content (oxalates and tannins) of the *Hibiscus sabdariffa* with different fertilizer levels.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Vegetables

Vegetables are mostly annual crops belonging to the group of plants called horticultural crops which are diverse in nature, (Taura and Habibu, 2009). On the bases of the edible parts, leafy vegetables are the most common, next to fruit vegetables in terms of human consumption. Leafy vegetables are usually harvested green for human consumption. When fresh they have a high water content of about 80%. Like many vegetables, leafy vegetables are important source of food for humans. Vegetables possess a high content of minerals and vitamins. Fresh and minimally processed vegetables provide most of our daily requirements for vitamins, minerals and fibre. Their role in reducing the risk of lifestyle associated illnesses such as heart disease, diabetes and cancer has resulted in a further increase in their desirability and consumption. In order to benefit significantly from these health properties, the World Health Organization (WHO) recommends an intake of 400g, or five to nine portions, of fresh fruits and vegetables per day (Matthews, 2006).

The World Health Organization has issued reports claiming that correct fresh vegetable intake alone could save 2.7 million lives a year and that 31% of heart disease cases are due to an insufficient intake of such foods (Johnston *et al*., 2006). According to Matthew (1985), green leafy vegetables are considered as good source of vitamins, minerals such as copper and iron.They are valued mainly for the high carbohydrate, vitamins and mineral contents (Robinson, 1990).

Parr and Hornick, (1990), explained that, with increasing cost of mineral fertilizers and concern about their future availability, there is renewed interest in organic recycling to improve soil fertility and productivity. Organic compost wastes may be utilized in the soil as

a source of nutrients for crop production. Increasing compost application in the newly reclaimed soils significantly increased both the dry matter production and yield of fruits (Abou El-Seoud *et al.*,1997). Sowicki (2003) found that organic compost addition significantly increased sunflower dry weight, seed yield, oil content, carbohydrates and major elements (NPK) as well as Fe, Mn, Zn, Co and Ni content of seeds. Cobalt is also required in low levels for maintaining high yields of tomato (Runner *et al* 2003), squash (Atta – Aly, 1998), groundnut (Basu *et al.*, 2006), parsley (Laila and Nadia 2002), sweet potato (Nadia and Hala 2008), Canola (Nadia, 2010) and peppermint (Aziz, *et al.*, 2011). Organic fertilization is a very important method to provideplants with their nutritional requirements without havingdetrimental impact on the environment.

Ahmed *et al.*,(1998) studied the effect of active dry yeast and farmyard chicken and animal manure on roselle plants, with 2gm/liter during the vegetative, flowering and fruiting stage,a significantly improved growth yield of calyces and active substances were observed. Chicken and animal manure which were applied at levels 14, 18 and 27m^3 /feddan enhanced growth, quantity and quality of calyces, particularly at the low rates. Nabila and Aly (2002) found that both chicken manure and animal manure enhanced plant height, number of branches and fruits, mass production of the plants, fresh and dry weights of sepals per plant and per feddan.

An increase of anthocyanin content was observed in plants treated with combination of chicken manure mixture of micronutrients while the acidity was reduced. They also added that the total and soluble carbohydrates in sepals as well as ascorbic acid positively response to all treatments. El-keltawi *el al.,* (2003) reported that, in a field experiment in Sohag and Assuit, application of the organic sugarcane wastes (12ton/fed) resulted in a slight increase in the protein and phosphorus content of sepals of roselle plant grown in Sohage. Laila and Nadia (2002) reported that, cobalt at 25 mg/kg soil had shown a positive effect on parsley

plant growth yield, minerals composition (N, P, K, Mg, Ca, Mn, Zn and Cu), chlorophyll and L-Ascorbic acid content. Aziz *et al.,* (2007) described that, cobalt at 20 mg/kg soil possess a synergistic effect on status of N, P, K, Mn and Zn in roselle leaves and calyces.

2.2 Minerals Nutrients

2.2.1 Calcium

Green vegetables (including *Hibiscus sabdariffa*)are one of the major sources of calcium in diet. Most of the calcium in the body is present in the bones and teeth; only a small amount (1%) is present in blood/body fluids. occupies a pivotal role in cell signal transduction.The availability of calcium is reduced as it binds to form insoluble calcium phytate/oxalate. In plants, calcium is primarily associated with the pectic materials. It is believed to have a major influence on the rheological properties of the cell wall and, consequently, on the texture and storage life of fruits and vegetables. Recommended Dietary Allowances for calcium ranges from 400-1000 mg per day depending on age, sex and physiological conditions. There is also evidence linking hypertension with calcium deficiency (Appel *et al*., 1997; McCarron and Reusser, 2001).

2.2.2 Iron

Iron is a constituent of the haem complex, a naturally occurring plant chelate involved in electron transfer in a number of important plant enzymes (Mengel and Kirkby, 1982). The plant plastid stroma may contain deposits of phytoferritin, a storage form of iron similar to the ferritin of animal cells. Phytoferritin occurs almost exclusively in plastids and most abundantly in the plastids of storage organs (Briat and Lubreaux, 1997). In green vegetable leaves, there is a good correlation between iron supply and chlorophyll content. Inadequate iron nutrition results in abnormal chlorophyll development, so that deficiency begins as an interveinal chlorosis on younger leaves resulting in prominent green veins. The resultant

reduction in photosynthetic capability also reduces the weight and area of affected leaves. Descriptions of causes of iron deficiency have been extensively reviewed for horticultural crops (Korcak, 1987).

Adult users of lower-fat foods consume more nutrient-dense diets, with higher intakes of iron (Kennedy et al., 2001; Sigman-Grant et al., 2003). Adult users of lower-fat foods consume more nutrient-dense diets, with higher intakes of iron (Kennedy *et al*., 2001). The predominant source of iron in the American food supply is grain products, followed by the meat, poultry and fish group. Between 1909 and 1919, the vegetable group furnished an average of 18% of the iron in the food supply, but in 2004 that share dropped to an average of 10% (Sigman-Grant *et al*., 2003).

2.2.3 Magnesium

Generally, magnesium levels are significantly higher in vegetables than in fruits, but nuts are good sources of this nutrient. Dry fruits and legumes are the food groups that rank higher in magnesium content (Jodral-Segado *et al*., 2003). This is the third most abundant mineral in the human body, approximately seventy percent of which is located in the bone. The single most important and most common mineral deficiency is probably magnesium. Like calcium, magnesium must be constantly supplied to maintain optimal body function. The possible reasons for a deficiency include; kidney or parathyroid disease, high blood pressure.Mixed users, who are more likely to consume higher intakes of grains, fruit and milk products, were found to have higher magnesium densities than high-fat users, who consume significantly more servings of meat and higher levels of discretionary fat (Sigman-Grant *et al*., 2003).

2.2.4 Sodium

An essential mineral found predominantly in blood serum, and extracellular fluid, as well as in bone. It functions synergistically and inversely with potassium to assist in pH balance, and helps keep other blood minerals soluble. It is essential for hydrochloric acid production and very involved in overall adrenal function. Sodium and potassium generally determine the body's electrolyte balance, which regulates water levels. Eating a lot of salty food (sodium) disrupt this balance, not only produces high blood pressure, but also affects neurotransmitter levels. Thus, the relative contribution of vegetables to reasonable amount of sodium in the food supply is overstated (Hiza and Bente, 2007).

2.2.5 Potassium

Potassium (K) a potassium-rich diet contributes to lower blood pressure, blunting the effects of salt (Salunkhe *et al*., 1991). Inadequate levels of potassium intake have long been associated with higher blood pressure (McCarron and Reusser, 2001). Potassium also regulates heartbeat, assists in muscle contraction and is needed to send nerve impulses and to release energy from fat, carbohydrates and protein. Nutrients and phyto-chemicals from fruits and vegetables, including potassium, may be jointly responsible for an apparent reduction in cardiovascular disease risk (Ignarro *et al.*, 2007). Potassium is a systemic electrolyte and is essential in co-regulating ATP with sodium. Potassium favorably affects acid–base metabolism, which may reduce the risk of developing kidney stones (Zerwekh *et al*., 2007), and possibly decrease bone loss with age, in women consuming high intakes of potassium, magnesium, zinc and vitamin C emphasizes the importance of considering the impact of variation in other nutrients when focusing on a particular mineral (Cohen and Roe, 2000). In fact, up to 11 different groups of compounds (vitamins, minerals, antioxidants and others) in fruits and vegetables could influence bone health (MacDonald, 2007).

2.2.6 Manganese

Manganese (Mn) Manganese is a key component of enzyme systems, including oxygen-handling enzymes. Isa trace mineral important in many enzymes and the metabolism of many vitamins as well in the synthesis of fatty acids and cholesterol, protein, carbohydrates, and fat metabolism , as well as sex hormone production, bone and connective tissue, health, and nerve and brain function. It supports brain function and reproduction and is required for blood sugar regulation. In addition, it is part of bone structure. Manganese is a cofactor in function of antioxidant enzymes, such as the mitochondrial superoxide dismutase. In plants, manganese atoms appear to undergo successive oxidations to yield a strongly oxidizing complex that is capable of water oxidations during photosynthesis. Also like magnesium, manganese is required in enzyme reactions involving carbon assimilation. Chloroplasts are most sensitive to manganese deficiency (Soetan *et al.,* 2010).

2.2.7 Copper

Copper (Cu), plays an important role in the oxidative defense system. In fact, oxidative stress is a characteristic of copper deficiency (Uriu-Adams and Keen, 2005). Copper is necessary for the formation of hemoglobin and is required for the function of over 30 proteins, including superoxide dismutase, ceruloplasmin, lysyl oxidase, cytochrome c oxidase, tyrosinase and dopamine- β -hydroxylase (Arredondo and Nuñez, 2005). Deficits of this nutrient during pregnancy can result in gross structural malformations in the fetus, and persistent neurological and immunological abnormalities in the offspring (Uriu-Adams and Keen, 2005).

2.2.8 Zinc

Zinc (Zn) is a pervasive microelement that plays a catalytic or a structural role in more than 200 enzymes (e.g. carboxypeptidase, liver alcohol dehydrogenase and carbonic

anhydrase) involved in digestion, metabolism, reproduction, and wound healing. Zn is a cation with various coordination possibilities and several potential geometries. Thus, it is easily adaptable for different ligands. The main role of structural Zn^2 in proteins is to stabilize tertiary structures. In addition, zinc has a critical role in immune response, and is an important antioxidant. Zinc activates a number of plant cell enzymes (Romheld and Marschner, 1991), but only a few of them (i.e. alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase, RNA polymerase) contain the micronutrient. Zinc can affect carbohydrate metabolism because different Zn-dependent enzymes participate in biochemical reactions involving sugars. A comprehensive review of soil, plant and factors associated with zinc nutritionalvalue in horticultural crops has been developed by Swietlik (1999). Fruits and vegetables account for only 1.2% and 6.4%, respectively, of the zinc in the American food supply (Hiza and Bente, 2007). As is the case for magnesium, zinc intakes may be below the adequate levels for both adults and children (Sigman-Grant *et al.*, 2003).

2.2.10 Fiber

It is difficult todefine fiber due to the fact that not all fiber consists of the same structures. Soluble andinsoluble fibers categorize fiber by the ability to hold water. Fiber is "the amount of plant material remaining after treatment with digestive enzymes and reduction with acid and alkali". Fibers that do not dissolve in hot water are referred to as insoluble fibers. Cellulose, hemicellulose, and lignins are insoluble fibers that accelerate the gastrointestinal transit time, increase fecal weight to promote bowel movements, slow starch hydrolysis, and delay glucose absorption (Whitney and Rolfes, 1999). Soluble fibers are those thatdissolved in hot water,examples hemi-cellulose, pectin, gums, and mucilages. Soluble fibers delay gastrointestinal transit time, delay glucose absorption, and lowerblood cholesterol. Several United States governmental and private organizations recommend the

increase in*dietary* fiber due to the research that has proven fiber to have beneficial health effects. Fiber's physiological functions improve the health of children and adults of all ages. The hypolipidemiceffects of lowering the lipid levels in the blood are valuable for the prevention of atherosclerosis.The hypoglycemic effects are very advantageous for slowing the carbohydrate absorption rate fordiabetic individuals. Constipation, cancer, and other gastrointestinal problems can be preventedby sufficient amounts of fiber. Also, individuals struggling with weight maintenance have theability to use fiber for its abilities to delay gastric emptying (Groff and Gropper, 2000).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Lists of Apparatus/Equipment

3.1.1.1 Equipment

Atomic absorption spectrophotometer, Flame Emission Spectrophotometer, Centrifuge, Water bath, Hot air oven, Soxhlet extractor, Fiber cap, Heating mantle, Hot plates, Digestion block, Muffle furnace, Analytical balance, Refluxing unit.

3.1.1.2 Apparatus/glasswares

The following were the glasswares used; volumetric flasks, conical flasks, measuring cylinders, filter papers (Whatman No.1 and No. 44), crucibles, petri dishes, distillation flasks, pipette, burettes. All the glasswares and containers were washed using 3% detergents and sucked in 2 % HNO_3.

3.1.2 List of reagents used

Perchloric acid 60% (v/v).

Conc. Nitric acid.

Conc. Sulphuric acid.

Petroleum ether (40 - 60^{o}C bp)

Boric acid 2% (w/v).

NaOH 40 % (w/v).

0.01 M HCl
0.02 M HCl

$KMnO_4$

3.1.3 Preparation of reagents

3.1.3.1 Folin-denis reagent

Sodium tungstate (50 g) was weighed into 375 cm^3 of distilled water, followed by addition of 10g phosphomolybdic acid, and then orthophosphoric acid (25mls) added and mixed together. This was prepared a fresh before use.

3.1.3.2 Sodium carbonate (17%)

Sodium carbonate (19 g) was dissolved in 50cm^3 of distilled water and diluted to mark of 100 cm^3 volumetric flask with distilled water.

3.1.3.3 Mix indicator

Exactly 0.2g of methyl blue was weighed into 100 cm^3 flask, also 0.4g methyl red was added into another 100 cm^3 flask. Both were dissolved using ethanol and mixed in 200 cm^3 volumetric flask thoroughly.

3.1.3.4 Ammonium acetate(1M)

Exactly 300ml of waterwasadded into a large aspirator,where 575 $cm^3$of glacial acetic acid added,then 600 cm^3 of 0.880 NH_3 solutions also added and mixed, then diluted to 10 L and thoroughly mixed.

3.1.3.5 Ammonium fluoride

NH_4F (1M);exactly 37g of NH_4F wasdissolved in distilled water andmade up to 1 L (stored in a polyethylene bottle).

3.1.3.6 Reagent "A " solution

a) Extracting solution

Exactly 15 cm^3 of 1M NH_4F and 25ml 0.5N HCl were added to 460 cm^3 of distilled water.

b) Ammonium molybdate*(*$(NH_4)_6\ MO_7O_2H$)

Exactly 12g of $(NH_4)_6$ MO_7O_2H was dissolved in distilled water to made up to 250 cm^3 volume.

c) Potassium antimony

Exactly 0.2908g ($KS_6OC_4K_4O_6$) was dissolved in (100 cm^3) of water and made to the mark

d) H_2SO_4(2.5 M)

Exactly 148 ml of conc. H_2SO_4 was pipetted into (1000 cm^3) volumetric flask and then diluted to 1000 $cm^3$with distilled water.

The reagent A solution, was prepared by mixing the solutions a, b, c and d in a 2 L volumetric flask and made up to the volume with distilled water.

3.1.3.7Reagent B

Exactly 1.056 g of the ascorbic acid was dissolved to 200 ml of reagent A (called reagent B), which was prepared a fresh before used.

3.1.3.8 Catalyst

Exactly 50 g of the anhydrous copper sulphate was weighed and 0.5g of selenium powder was added mixed and grounded to powder.

3.1.4 Preparation of aqueous solutions

3.1.4.1 Tannic acid standard

Tannic acid (0.05 g) was dissolved into 500 cm^3 volumetric flask and diluted with distilled water to mark (1 cm^3=0.1mg tannic acid of the stock solution). This was prepared fresh.

3.1.4.4 Sodium standard solution

Stock solution (100ppm Na); Dried NaCl (0.2542 g) was dissolved and made up to 1000 cm^3 mark of the volumetric flask with distilled water.

Working standards:the working standards were also prepared by appropriate dilution of the stock solution. A concentration range of 0.5 – 5ppm Na were prepared and stored in plastic vials.

3.1.4.5 Calcium standard solution

Stock solution (1000ppm Ca); prepared by dissolving 2.4973g of dry Calcium carbonate in about 200 cm^3 of water containing 5ml of conc. H_2SO_4, then heated to drive off CO_2, cooled and made up to 1 liter mark.

Working standard; a range of 10 – 50ppm were prepared from stock solution, where1% $LaCl_3$ (w/v) added and made final dilutions. Blank also prepared containing same amount of Lanthanum concentration to overcome anionic interference.

3.1.4.6 Zinc standard solution

Stock solution (1000ppm Zn); exactly 0.4398g $ZnSO_4.7H_2O$ was dissolved in distilled water and made up to 1L mark.

Working standards; the serial dilutions of the working standard were prepared from stock solution and made a range of 0.4 – 2ppm Zn for the calibration curve.

3.1.4.7 Iron standard solution

Stock solution (1000ppm Fe); 0.100g of clean untarnished Fe wire was dissolved in about 10cm^3 of warm 10% H_2SO_4 cooled and diluted to liter.

Working standards; the working standards were prepared from the stock solution by taking the appropriate volume to made ranges of 4 – 20 ppm for the calibration curve**.**

3.1.4.8 Copper standard solution

Stock solution (100ppm Cu); 0.3930g of $CuSO_4.5H_2O$ was dissolved in distilled water, shaking and made to 1L mark as a stock solution.

Working standards; the working standards for the calibration curve also prepared, by serial dilution of the stock solution at a range of 2 – 10ppm Cu.

3.1.4.9 Magnesium standard solution

Stock solution (100ppm Mg); 1.0136g of $MgSO_4.7H_20$ dissolved in distilled water containing about $1cm^3$ conc H_2SO and diluted to 1L.

Working standard; stock solution diluted and make a range of 0.2 – 1.0ppm Mg. where sufficient 1% $LaCl_3$ (w/v), also added and made final dilutions. Blank wasalso prepared containing equivalentof La concentration to overcome anionic interference.

3.1.4.10 *Manganese standard solution*

Stock solution (100ppm Mn); 0.4050g of $MnSO_4.4H_2O$ dissolved in distilled water containing $1cm^3$ conc. H_2SO_4 and diluted to 1L mark with distilled water.

Working standard; the stock solution diluted by pipetting the required volume and made a working standard range of 0.6 to 3ppm.

3.1.4.11 Potassium standard solution

Stock solution (1000ppm); 1.0968g of dried KCl was dissolved in distilled water and made up to 1L mark.

Working standard; suitable range of 20 to 100ppm K prepared from the stock solution, and made the serial working standard solution.

3.1.4.12 Lead standard solution

Stock solution (1000 ppm); exactly 0.1599g of $Pb(NO_3)_2$was dissolved in $20cm^3$ 1% (v/v) HNO_3 and diluted to 1L with distilled water.

Working standards; the working standard were prepared by serial dilution from the stock solution at a ranged of 2-10ppm Pb.

3.1.4.13 Phosphorus standard

Stock solution (1000ppm P); 0.4393g of dried KH_2PO_4 was dissolved in volumetric flask (1000 cm^3) with distilled water and diluted to mark.

Working standard; Set of standards P solutions also prepared containing 0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0ppm P (Allen, 1974).

3.1.4.14 Calibration curve

The calibration curve for element was prepared by serial dilution of the stock solution to a precise range as mentioned earlier in the preparation of working standard solutions. The standard and blank solutions were aspirated first before the optical reading in the instruments (Atomic Absorption and Flame Emission Spectrophotometers), for the establishment of the calibration curve and blanking at the following wave length; 589 nm for Na, P(883), Pb(283.3), K(766), Mn(279.5), Mg(285.2), Cu(3248), Fe(248.3), Zn(213.8) and then Ca(422.7nm) respectively (Allen, 1974).

3.2 Methods

3. 1.1 Description of the studyarea

The study was carried out at research farm of Kano University of Science and Technology, Wudil,sited at Gaya Local Government.The samplingwas conducted between July and November, 2014. The geographical coordinates of this farm is; latitude $11^{o}51'28.05''N$, longitude $8^{0}59'11.76''E$, and altitude of 430m above sea level. The area has mean annual rain of about 800mm with relative humidity of 75% at rainy season and the annual average temperature ($25^{o}C$- $26^{o}C$).The plant was grown in Gaya farm and the analysis was conducted atthe Animal Science Laboratory, Biological Science Laboratory, Multi-user Laboratory and Soil science Laboratory, ABU Zaria.

3.1.2 Experimental Design

The experimental design, consisted of a complete randomized design block with three replications, the distance between two consecutive blocks was 1m and 2m between replicates. The block was 3.6m long and 4.9m wide. All plots consisted of 6 rows of plants with 0.7m apart and 0.6m intra spacing within rows. Four central rows were sampled throughout the period.

3.1.3 Crop establishment

Hibiscus sabdariffa (*L.*) was the plant variety used for this research, the poultry manure wasapplied uniformly after land preparation before sowing at a rate of 10 ton/ ha asa first treatment (T1) and the seeds were directly sown by digging in furrows exactly same day after rainfall together with mineral fertilizer (NPK-15-15-15) at a rate of (75 and 100 kg)/ha assecond and third treatments (T2 and T3). The thinning was done to an intra-row spacing three weeks after planting. Shallow cultivation was observed; using hoe and hand pulling to keep the plots weed free, for about three times (Grubben and Denton, 2004).

3.2.1 Quality assurance

3.2.1.1 Sampling and sample processing

a) **Plant sample Collection**

The plant and soil were sampled at the following three stages of *Hibiscus sabdariffa's* growth;

Stage I: the vegetative stage of *Hibiscus sabdariffa's* growth at 25 days after sowing,

Stage II: flowering stage of *Hibiscus sabdariffa's* growth at 75days after sowing and,

Stage III: maturity/fruiting stage of *Hibiscus sabdariffa's* growth at 115 days after sowing but, here calyx (fruit) included.

The plant were randomly selectedby uprooting across the stages (SI, II, III) for leaves sample, five (5) plants were randomly selected from each plot andthe calyx sampled at maturity stage (SIII). The soil was also sampled togetherwith plant sample at a deep of 20cm.

b) Soil sample Collection

A randomsampling technic wasapplied in the soilsampling from the soil surface at 20 cm depth across the stages of the plant growth (SI, II, III) and control, equal volume of the soil were obtained in each sub-sample and mixed to made composite sample per plot, where the clods broken and mix thoroughly. The large rocks, pieces of sod, earthworms,were also removed and put the soil into the fresh polyethylene bag and labelleclearly. All information about the samples recorded, and each sample was given a laboratory number(George *et al.*, 2013).

c) Sample processing

Preparation of the plant sample

The plant samples were washed with tap water followed by distilled water and then separated into organs and dried at room temperature (under shed).

Preparation of soil samples

The soils were allowed to dried at a room temperature (< 35 °C), where the dried samples are ground with a wooden pestle and mortar to provide maximum surface area. The entire sample were powdered and passed through 2mm sieve and then put in a fresh polyethylene bag and labeled (George *et al.*, 2013).

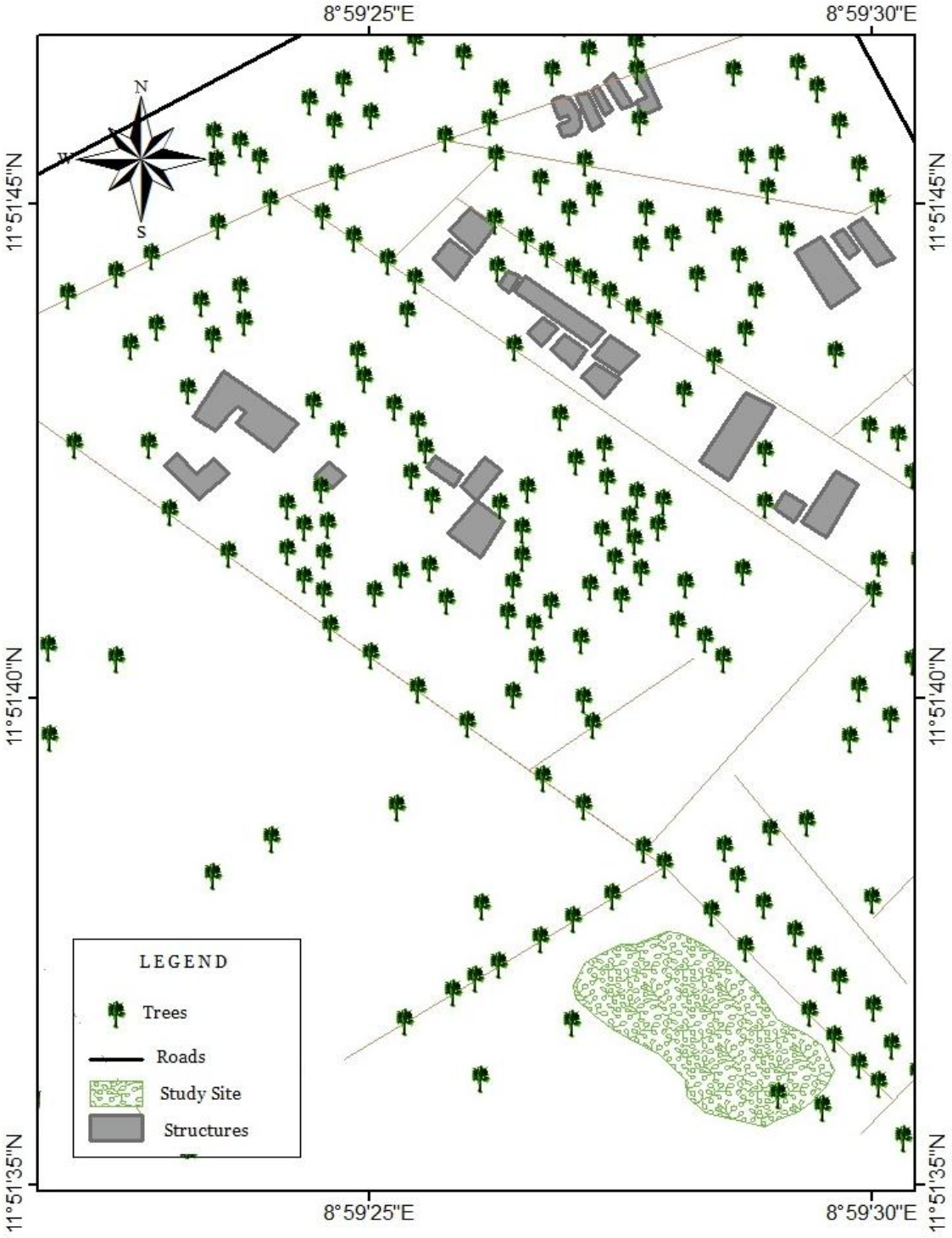


Figure 3. 1: Map of KUST Research Farm, Gaya indicating the sampling site

SOURCE: GIS Unit, Dept. of Geography KUST, Wudil (2017)

3.2.3 Proximate, minerals and anti-nutrients analysis of the *Hibiscus sabdariffa*

Proximate compositions (moisture, ash, crude fat, crude fiber, crude protein, calorific and carbohydrate contents), anti-nutrients(tannin and oxalate and then metals (sodium, calcium, magnesium, manganese, iron, zinc, copper, potassium and lead)were determined from the plant leaves and calyx samples as follows;

(a) Determination of moisture content

Fresh sample (5 g) was weighed and placed in a clean dry moisture dish and the weight of the sample and dish taken. These were placed in a hotair oven and the temperatures adjusted to 105^0C. The samples were dried for 3 hours and cooled in desiccators and weighed. The procedure was repeated several times until a constant weight was obtained. The moisture content in the samples calculated using the formula:

$$\% \text{ moisturecontent} = \frac{(W2-W1)-W3}{(W2-W1)} x100 \text{(AOAC, 1995).}$$

Where: W_1 = Weight of empty crucible, W_2 = Weight of empty crucible + sample, W_3= Weight of W_2after drying.

(b) Determination of ash content

Fresh sample (5 g) wasweighed into a cleanedpre-weighed crucible, and charred by heating in a fume hood till smoking ceased. The charred samples were then transferred to a muffle furnace and temperature increased gradually to 550^oC. The samples were then completely ash. Temperature was reduced, samples removed and cooled in a desiccator before weighing. The amount of ash was therefore calculated using the formula:

$$\% \text{ Ashcontent} = \frac{(W3)-W1}{(W2-W1)} x100 \text{(AOAC, 1995).}$$

Where: W_1 = Weight of empty crucible W_2 = Weight of empty crucible + sample, W_3= Weight crucible + ash.

(c) Determination of protein content

Exactly 1 g of sample was weighed into a digestion flask together with a combined catalyst of 5 g potassium sulphate and 0.5 g of copper sulphate and 15cm^3 of sulphuric acid. The mixture was heated in a fume hood till the digest color turned blue.The digest was cooled, transferred to 100 cm^3 volumetric flask and topped up to the mark with de-ionized water. A blank digestion with the catalyst was also made. Exactly 10 cm^3 of the diluted digest transferred into the distillion flask and washed with distilled water. 15$cm^3$of 40% NaOH added and this also washed with distilled water. Distillation was done to a volume of about 60 cm^3 distillate. The distillate then titrated using 0.02 M HCl to an orange color of the mixed indicator, which signified the end point (AOAC, 1995). The nitrogen in the samples calculated as;

Nitrogen content(%)= $\frac{(V1-V2)\ X\ N\ X\ F}{(V\ X\ S)}$X100

Where:

V_1 = the titre for sample in ml, V_2 = titre for blank in ml, N= normality of standard HCl (0.02), F= factor of std HCl solution, V= volume of diluted digest taken for distillation (10 ml), S= weight of sample taken for distillation (1 g).

The protein content was then calculated as:

Protein contents (%)= Nitrogen x protein factor (6.25) (AOAC, 1995).

(d) Determination of fat content

A 250ml boiling flask washed, dried in anoven (at 105-110°C) and then cooled in a desiccator.About 2g of the sample (W_2) was weighed, put in theextraction thimble and then plugged. It was then placed backin the soxhlet apparatus. The cleaned boiling flaskalso filled to 2/3(cm^3) withpetroleum ether. TheSoxhlet apparatus then assembled and allowed toreflux for about 6 hours which then completed the extraction. The thimble was carefully removed

and dried at 80°C for 30 minutes in the oven (Gallenkamp, VWR 1730) and cooled in a desiccator and finally weighed (W_3) AOAC(2010). The fat content calculated as follows;

Fat contents(%) = $\frac{W2 - W3}{W1}$X100 (AOAC, 2010)

Where:W_1 = Weight of empty thimble, W_2 = Weight of empty thimble + sample, W_3= Weight of W_2 after extraction.

(e) Determination of crude fiber content

2g (W_1) of sample was weighedusing analytical balance and put in a 250 cm^3 beaker, 100ml of0.12M $H_2SO_4$was added boiledfor 30 minutes and filtered. The filtrate washed with boiling water until the washing was nolonger acidic. The filtered sample transferred backand boiled for another 30 minutes now with 100 cm^3 of 0.012M NaOH solution, filteredwith hot water, followed by methylated spirit three times. The residue wasthen transferred into a crucible and dried in theoven for 1 hour. The crucible plusthe content was cooled in a desiccator and then weighed (W_2). The residue was then taken into the furnace for ash at 600°C for 1 hour. The ash samplewas then removed from the furnace and put into the desiccator for1 hour weighed (W_3).

The percentage crude fiber was calculated thus:

Crude fiber contents (%) = $\frac{W2 - W3}{W1}$X100(AOAC,2010).

Where:W_1 = Weight of original sample**,** W_2 = Weight of crucible and residue before ash**,** W_3 = Weight of final ash sample.

(f) Determination of carbohydrate content

Thecarbohydrate content was determined by difference in proximate analysis (AOAC, 2010), as described by Oyenuga (1968) using the equation below;

Carbohydrate contents (%) = [100 –(%Moisture + %Ash + %Protein +%fat+%fiber)]

(g) Calorific value

The calorific valueestimated (in Kcal) by multiplying the percentagesof crude protein, crude lipid and carbohydrate contents obtained beforehand in proximate analysis by the recommended factors 2.44, 8.37 and 3.57 respectively (Asibey-Berko and Tayie, 1999)

(h) Determination of Tannin content

The ground sample(0.1 g)was weighed into 100 cm^3 conical flask, where 50ml of water added,mixed and boiled for 1 hour ona hot plate. The extract was filtered whilst warm through a No.44 Whatman filter paper into a 50 cm^3 volumetric flask. The filtratethen diluted to volume after cooling by running the water onto the extract residue on filter paper.Blank also prepared. The color was developed by pipetting the filtrate sample aliquot (5 cm^3) into another fresh volumetric flask(50 cm^3), and also tannic acid standard prepared in the following order (0, 0.5, 1, 1.5, 2, 2.5and 3cm^3), present 0 – 3 mg of tannic acid and then put into 50cm^3 volumetric flask.At this point, samples and standard solutions were equally treated by additionof distilled water into the flask (two-third) full. 2.5 $cm^3$of folin-denis reagentadded followed by 10 cm^3 of Na_2CO_3 solution,and then diluted to mark and mixed. The mixture then kept in a water bath at 25^{o}c for 20 minutes, where the optical density of the standard read at 760nm forthe calibration curve and then sampleswith blank reading measured.

Calculation for tannin content;

$$\text{Soluble tannin (\%)} = \frac{\text{C (mg) x extract volume (cm 3)}}{\text{10 x aliquot (cm 3) x sample weight (g)}}$$

Where C (mg) = sample reading – blank

(i) Determination of oxalate content

According to the AOAC (1999) method, oxalate was determined by weighing 0.1g of sample and mixed with 30 cm^3 of 1M HCl.Each mixture was then shaken in a water bath at 100^0C for 30minutes. To each extract, 0.5ml of 5% calcium chloride was added and mixed thoroughly for the precipitation of calcium oxalate. The suspension was then centrifuged at 3000rpm for 15 minutes to separate the supernatant. The pallets were washed twice with 2 $cm^3$of 0.35M NH_4OH and dissolved into a 0.5M H_2SO_4. The mixturewas then titrated with 0.1M $KMnO_4$ solution, while temperature wasmaintained at 60^oC. Theend point was reached as the faint violet color appeared and persisted for at least 15 seconds, and 1 cm^3 titer value equivalent to 2.2mg of oxalate.

(j) Determination of mineral composition

Digestionof plant sample for elemental analysis

Oven dried sample (0.20-0.50g) was weighed into a 100 cm^3 kheldahl flask.1ml of 60 % $HClO_4$wasadded, followed by 5 cm^3 HNO_3 and then 0.5 cm^3conc. H_2SO_4. The mixture was then swirled gently and digested slowly at moderate heat and increased later for 15 minutes after the white fumes appeared and then cooled. The digest wasthen diluted and filtered into 50 cm^3 volumetric flask using No.44 filter paper, and diluted to mark. The blank was also, obtainedin the same way with no sample included,and the digestswasused for the elemental analysis (Allen*et al*., 1974).

(i)Extraction of soil sample for elemental analysis

Exactly 10g of air dried sieved soil wasweighed into a 500 cm^3 container,250 cm^3 of M NH_4OACwasadded and shaken for 1hr on a rotary shaker the solution was then filtered through No.44 Whatman filter paper in polyethylene vials, wherethe first 20 to 30 cm^3extract transferred into a separate container and used for the determination of K element.The blanksample was also prepared and treatedsame way with extractant (Allen*et al*., 1974).

(ii)Elemental analysis

The solutions obtainedafter digestion for the plant tissue and extraction of the soil, were analyzedby AtomicAbsorption Spectrophotometer (A.A.S) at Multi-user Laboratory and Flame Photometer at Soil Science Laboratory, A.B.U, Zaria.

3.2.4 Determinationof physico-chemical parameters of the soil used

(a) Soil pH (1:1 soil in water)

20g of driedsoil sampleweighed carefully into the 50cm^3 beaker and then 20 cm^3 distilled water added. It was stirred with a glass rod and allowed to stand for 30 minutes to allow most of the suspended clay to settle out from suspension, and pH values were taken by dipping the electrode carefully. The result was reported as soil pH in water(George, *et al.*, 2013).

(b) Soil pH (1:1 soil in 0.1M$CaCl_2$ solution)

In this case 0.1M$CaCl_2$ solution was used the same way as done with water. A pre-calibrated HANNA pH meter (Model H1991000) was inserted into the slurry and the pH wasrecorded, as pH measured in 0.01M $CaCL_2$medium(George*et al.*, 2013).

(c) Determination of moisture content

Moisture content was determined according to the standard method described byAOAC, (1995) method. 5 g of fresh sample was weighed and placed in a clean dry moisture dish and the weight of the sample and dish taken. These were placed in a moisture oven and the temperatures adjusted to 105^0C. The samples were dried for 3 hours and cooled in desiccators and weighed. The moisture content in the samples wascalculated using the formula:

$$\text{Moisture contents (\%)} = \frac{(W2-W1)-W3}{(W2-W1)} x100$$

Where: W_1 = Weight of empty crucible, W_2 = Weight of empty crucible + sample, W_3= Weight of W_2after drying.

(d)Available phosphorus determination

Exactly 1g of air dried soil wasweighed into the 15cm^3 test tube, then 7 cm^3 of extracting solution was added, shaken with mechanical shaker where the suspension wascentrifugedat 2000rpm for 15 min and decanted into acid-washed volumetric flask. 2 $cm^3$of aliquot soil extract then pipetted into a 25 cm^3 volumetric flask, where the 10 cm^3 of distilled water also added, and then 4 cm^3 of the reagent B added,and made up to the mark, and allowed to stayed for 15 min for color development. The absorbance ofthe P wasread at 883nm. The blank and standard that contained the same volume of extracting solution were used for soil P determination (George, *et al*., 2013).

Calculation of P content;

$$\text{P content} = \frac{\text{R x Vx VD x DF}}{\text{V x 1 wt (g)}}$$

Where,

R= absorbance,VD= volume of digest (7),DF = dilution factor, Wt = weight of sample, V= vol. of aliquot.

(e) Determination of Total Nitrogen in Soil

2g of soil was weighed into Kjeldahl flask containing 20 cm^3 distilled water, swirled for a few minutes and allowed to stay for 30min. 3g catalyst and 20 cm^3 conc H_2SO_4 added and heated at a low temperature until the water expelled and frothing ceased, where the heat then increases and boiled the mixture for 5hour to obtain the cleared digests. The heating wasregulated during this boiling so that the H_2SO_4 condensed about half way up the neck of the tube, allowed to cooled and rinsed with distilled water. The digest was carefully

transferred into another clean flask and the residue was washed with 50 cm^3 distilled water four times each portion was transferred into the same flask and made to the mark. 10 cm^3 H_3O_3solution was added into the Erlenmeyer flask which was then placed under the condenser for distillation and 10ml of 40% NaOHwasslowly added, also 10 cm^3 digest wasadded in the flask that had already been attached to the distillation apparatus the temperature was raised to boils. The 50 $cm^3$of distillate (of the liberated NH_3) wascollectedand then 2-3 drops of mixed indicator wasadded and finally titrated from green to pink with standard 0.2M HCl(George, *et al.*, 2013).

$$\text{N content}(\%) = \frac{0.014 \text{ x VD x M x 100 xTV}}{\text{AD x sample wt (g)}}$$

Where, VD= vol. digest, M = Molarity of acid, TV = Titre, AD = aliquot of digest

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Compositions of *Hibiscus sabariffa*

Theresults of proximate analysisand anti-nutrients contents of*Hibiscus sabdariffa*atits growingstagesfrom three treatments of fertilizers: poultry manure (M), mineral fertilizer (F75 and F100) are presented in Figures 4.1 - 4.5.The mean content of moisture , ash, crude fat, crude protein, crude fiber, carbohydrate and calorific value of the plant cultivated using M, F75 and F100 fertilizerswere recorded in the ranges of 4.734±3.607 to9.319±2.545 M, 4.734±3.854 to7.524±0.449 F75, 2.469±2.203 to8.032±0.266 % F100 for moisture, respectively; 6.965±0.296 to12.411±0.600, 6.581±0.054 to 12.742±0.999, 7.0753±1.749 to 13.130±2.564 % for M, F75 and F100 ash content respectively; 3.069±0.320 to 20.910±2.132, 2.986±0.975to15.322±5.568, 3.335±1.312 to 18.426±0.704% crude protein was:9.431±0.679 to 15.455±2.428, 9.259±1.364 to 14.402±4.712, 11.242±5.369 to 30.710±0.054; crude fat: 10.160±0.308 to 13.233±2.422, 10.413 ±2.134 to 12.543±0.761, 11.373±3.106 to 10.867±5.452 % crude fiber: 32.366±5.555 to 60.440±1.177, 42.223±12.184 to 61.725±3.375, 41.960±7.657 to 56.497±1.591 % carbohydrate; 267.400±6.948 to 328.112±9.668, 276.445±16.411 to331.197±25.364, 288.276±35.572 to 305.367±22.426 kcal calorific value accordingly. Also, oxalate and tannin contents were found in the ranges: 5.931±0.618 to 14.423±0.387, 7.628±0.443 to 10.318±0.978, 6.028±0.845 to 11.904±2.168 % for M, F75 and F100 and the oxalate content recorded as: 2.408±0.672 to 5.230±3.012, 2.756±0.114 to 4.443±0.377, and 2.610±0.151to 4.190±0.346 % for tannin content respectively.

During the growing periods of *Hibiscus sabdariffa* (SI, SII, SIII and calyx), vegetative, flowering, maturity stages and fruit, the highest proximate and anti-nutrients

contents for each stage across the periods were recorded as: the moisture content (%) was: 6.989±1.053 % at SI, 6.990±0.920 % at SII, and 9.319±2.545 % at SIII and 8.032±0.266 % in the calyx. Similarly, the ash content (%) was also recorded as: 13.130 ±2.564 % at SI, 11.351±0.656 % at SII, 11.171±3.745 % at SIII and 7.075±1.749 % in the calyx. The crude protein (%) was found to be: 20.910±2.132 %, 13.117±1.815 %, 8.708±3.068 %, and 3.335±1.312 % accordingly in the SI, SII, SIII and calyx, respectively. The crude fat (%) also obtained to be: 30.710±0.054 % at SI, 11.242±5.369 % at SII, 15.652±3.922 % SIII and 14.094±2.272 % in the calyx. Meanwhile, crude fiber (%) recorded: 12.047±0.684 % in SI, 13.233±2.422 % SII, 11.373±3.106 % SIII and calyx 11.157±0.924 % respectively, the carbohydrate (%) recorded as: 42.223±12.184 % at SI, 55.431±5.286 % SII, 57.860±5.927 % SIII and 61.725±3.375 % calyx, and then calorific values (%) were: 288.276±35.572 kcal, 331.197±25.364 kcal, 302.668±30.606±4.851 kcal and 321.023 kcal) relatively. However,the oxalate content (%) at SI was: 9.978±2.359 %, 14.423±0.387 at SII,the 10.318±0.978 % SIII and 9.428±1.714 % in the calyx, and the tannin content recorded was found to be (%): 2.756±0.114 % SI, 2.857±0.371 % SII, 3.704±0.759 % SIII and 5.230±3.012 % from the calyx, respectively.

4.2 Minerals Compositions of *Hibiscus sabdariffa*

The levels of mineral compositions in the plant (*Hibiscus sabdariffa*) as presented in Figures 4.6 to 4.13 revealed that the mean concentrations of Na, K, Mg, Ca, Mn, Fe, Zn, Pb and Cu were in the ranges of 95.047±1.38 to 448.903 ±1.55 mg/kg for M, 53.628 ± 0.10to 155.342±0.91 F75, 81.136±0.12 to 339.001±1.84 F100 Na; and 10563.292±1.40to 14964.375±0.36, 7435.793±0.27 to 13718.690±0.42, 6173.347±0.56 to 17492.187±0.82 mg/kg K, for M, F75 and F100, 3801.449±1.72 to 7881.915±14.64,4569.806±5.15 to 7154.948±1.13, 2771.863±7.52 to 4927.246±3.08 to 59.995 mg/kg Mg for M, F75 and F100, 15298.459±24.99 to 33192.429±103.13, 13597.588±18.07to 29249.161±45.77,

22252.735±4.30 to 30995.241±34.88 mg/kg Ca; 518.251±0.11to 727.214±0.31, 463.280±0.69 to 857.639±1.05, 521.139±0.64to 928.713±0.55 Mn; 105.955±0.05 to 878.305±0.80, 122.934±0.10 to 922.508±2.49, 205.662 ±0.52 to 1377.294±6.54 mg/kg Fe; 30.689±0.02 to 53.942±0.15, 30.686±0.06 to 48.143±0.07, 21.376±0.05 to 59.995±0.09 mg/kg Zn; BDL to 23.607±0.04, BDL to 24.249±0.01,BDL to 26.624±0.06 mg/kg Pb; 4.105±0.01 to 18.571±0.04, 4.397±0.00 to 10.428±0.01, 5.427±0.01 to 13.222±0.04 mg/kg Cu, for the fertilizers M, F75 and F100 used in cultivation of the *Hibiscus sabdariffa (L)* respectively.

4.3 Physico-chemical parameters of the soil

Figures 4.17 – 4.23 indicated the soil phyico-chemical parameters for *Hibiscus sabdariffa's* grown under three conditions. The mean values for the macro elements such as nitrogen, phosphorus and potassium (NPK) at the stages of growth (SI-III) for *H. sabdariffa* were in the ranges of 210±70.000to 536.667±40.415, 256.667±40.415to 350±185.203,256.667±40.415 to 303.333±80.829 mg/kg, for N using M fertilizer, F75 and F100 fertilizers respectivelyat the stages of *H. sabdariffa*growth (SI-III). The least concentration of N recorded was at the control site. P concentration was in the range of 376.250±96.250 to 1023.833±417.392 M,414.250±169.069to 1153.583±625.866 F75, and 522.083±227.556 to 1080.667±958.329mg/kg when F100 used;and the mean concentration of K also ranged as: 2183.333±175.594 to 4533.333±3082.748, 1983.333±160.728to3016.667±189.297,and 1600±229.129to 3283.333±778.353mg/kg for the fertilizersstudied respectively. However, the percentage moisture contents were in the range of: 0.992 to1.677±0.262, 1.091±0.829 to 2.491±2.114,and 1.456±0.730 to 3.035±3.552% for M, F75 and F100 used respectively. The pH values in KCl and water medium were ranges from 6.277±0.0252to 6.640±0.0000, 6.303±0.3653 to 6.647±0.3086, 6.547±0.5235 to 6.527±0.2157for the pH in KCl medium and then 6.933±0.0252 to

7.513±0.1550,6.653±0.3009to 7.283±0.1877, 6.817±0.0851 to 7.193±0.0322for water medium when M, F75 F100 were used across the plant growth at SI-IIIrespectively.

4.4 Statistical Analysis

The data recorded for the proximate constituents, minerals composition, anti-nutrients and soil parameters were subjected to statistical analysis. The mean values were correlated using Pearson's correlation, and the results of the correlation analyses were presented in AppendixXIII and XIV. Some of the parameters were fairly correlated (>0.8 0r -0.8<). However, calorific values were found strongly correlated with carbohydrate content, soil potassium strongly correlated with crude protein, soil phosphorus and crude proteins were also strongly correlated with ash contents, the soil potassium were also strongly correlated to N and P concentrations, this clearly shows that the soil is highly fertile. However, soil nitrogen was correlated strongly with Cu and moderately correlated with Pb, which further shows that there was no base relation between N and Pb as soil nutrients. Moreover, Cu wasstrongly correlated to Pb concentration which basically indicates their level of association as heavy metals. The soil pH correlated with calorific value and carbohydrate content, which suggest that *Hibiscus sabdariffa* require higher pH to grow well. The tannin content was negatively correlated to crude protein, Na, Mg, Mn, Fe and Cu. More so, oxalate correlates negatively to minerals elements, K, Fe and Zn.

The analysis of variance (ANOVA) results are presented in (Appendix IV, VII and X). The result shows significance difference across the growing stages at ($p<0.05$). However, it was not significantly different among the treatments (at $p<0.05$), except the soil nitrogen (0.002). The ANOVA test for the analytes across the sampling period presented in (Appendix V, VIII and XI), indicated the effects of fertilizer treatments levels used at growing period of *H. sabdariffa* with regard to nutrients from plant and soil parameters at ($p<0.05$).

The concentrations of N, P and K in the soil, were significantly different among the stages of growth, and it was found to be insignificantly different among the treatments at ($p<0.05$). This naturally demonstrates the degree of changes that occur by leaching of nutrients as the plant was growing.

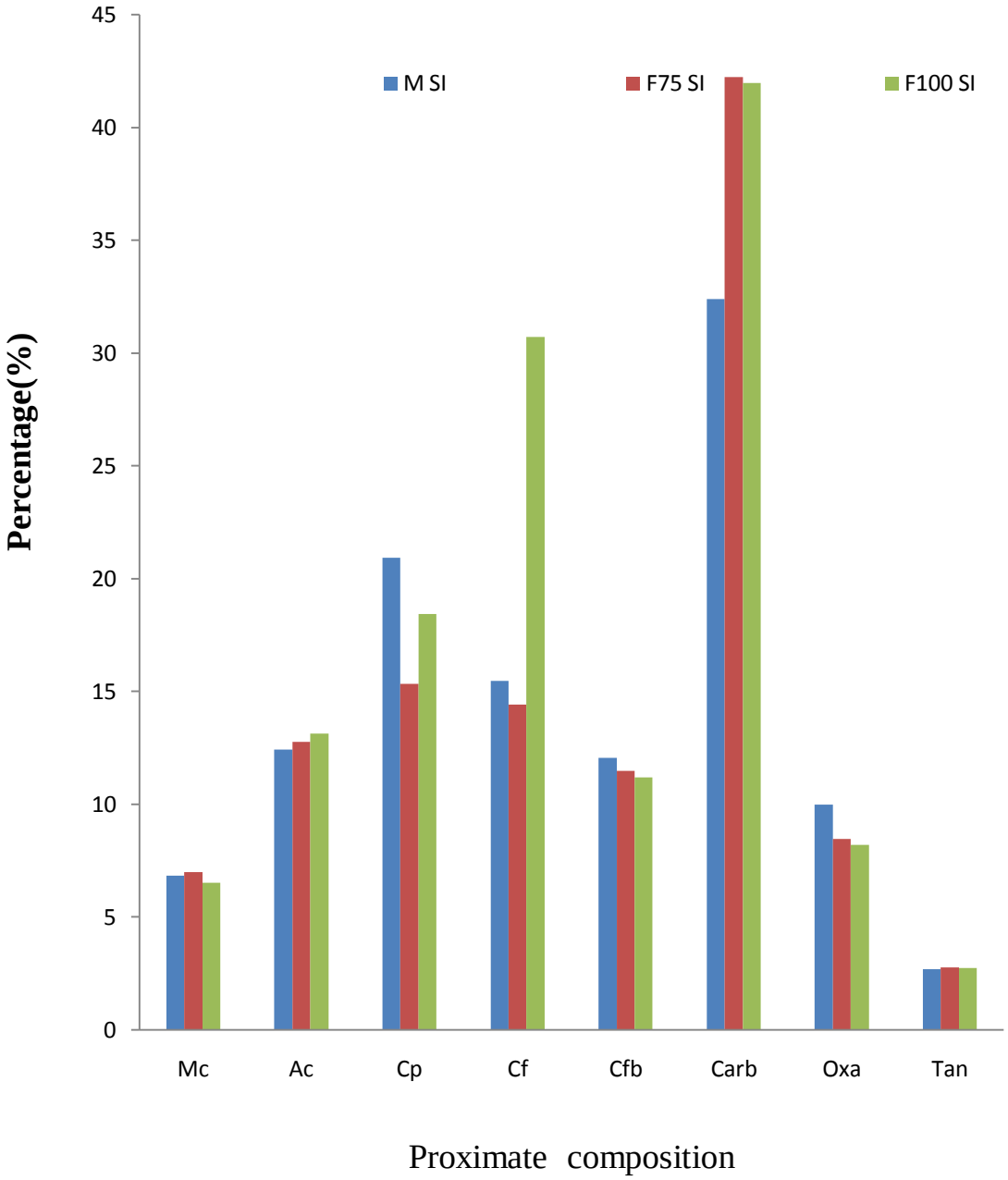


Figure 4.1: Proximate compositions of *Hibiscus sabdariffa* at vegetative stage of growth cultivated at different conditions(SI)

Key: M; manure, S; sample, F75; fertilizer 75kg/ha, F100; fertilizer 100kg/ha, Mc; moisture content, Ac; ash content; Cp; crude protein; Cf; crude fat; Cfb; crude fiber;Carb; carbohydrate, Oxa; oxalate, Tan; tannin

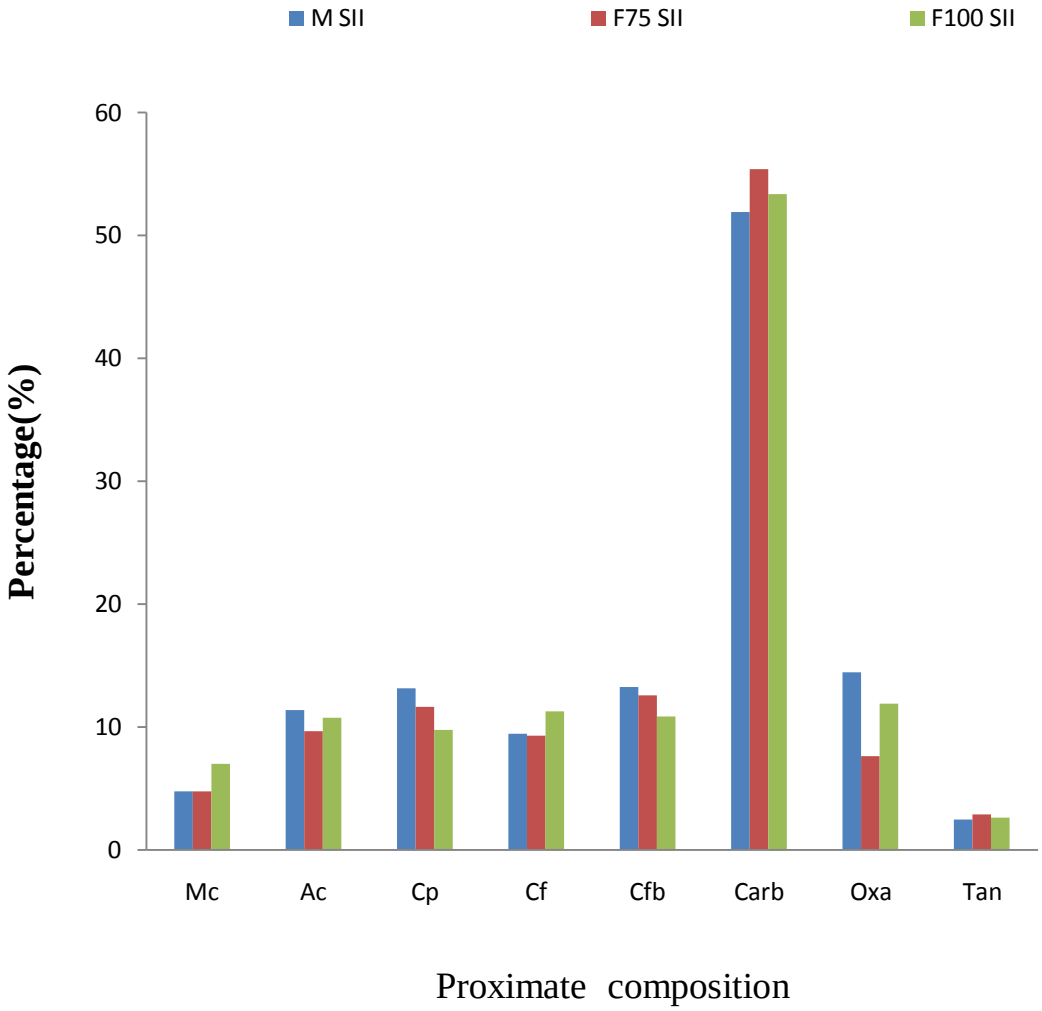


Figure 4.2: Proximate compositions of *Hibiscus sabdariffa* at its flowering stage of growth cultivated at different conditions (SII)

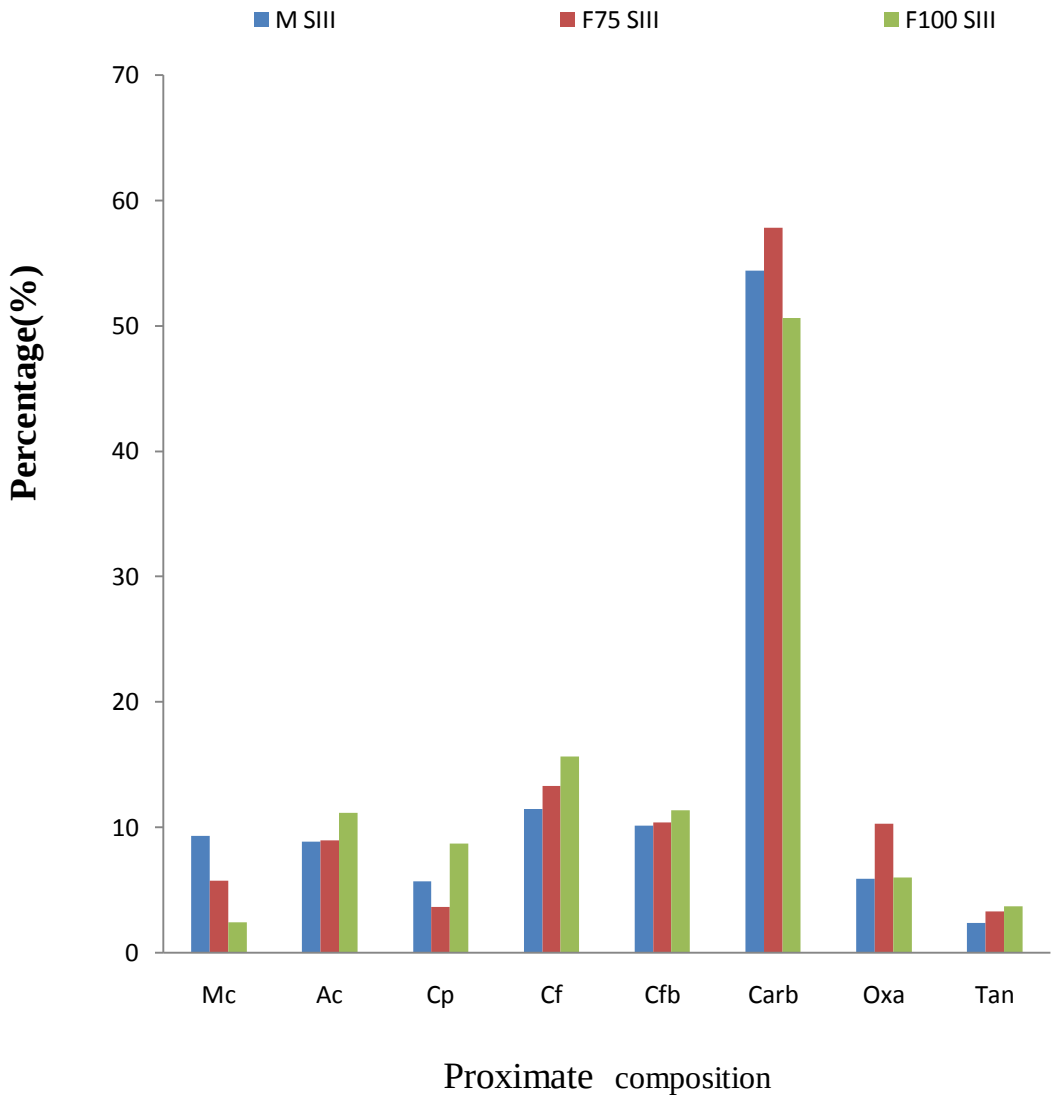


Figure 4.3: Proximate compositions of *Hibiscus sabdariffa*at its maturity stage of growth cultivated at different conditions (SIII)

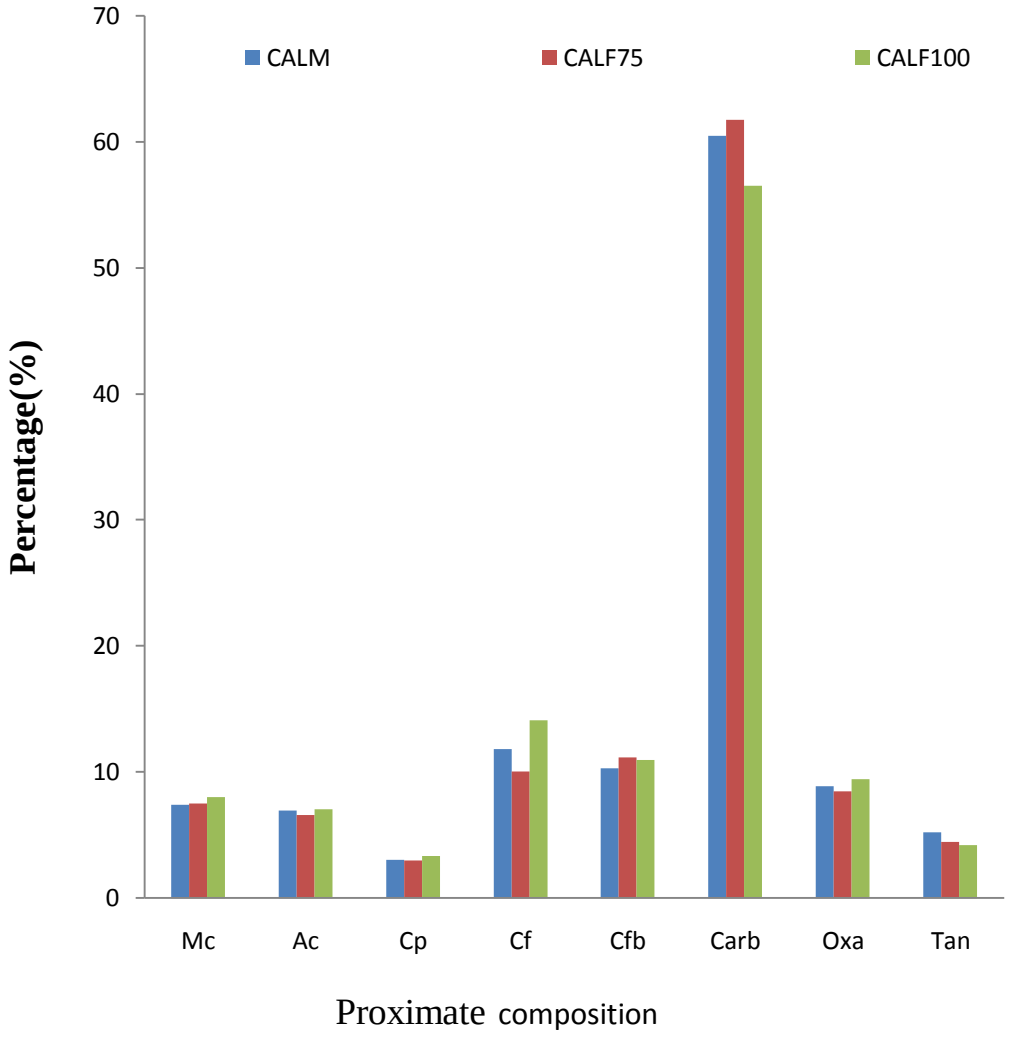
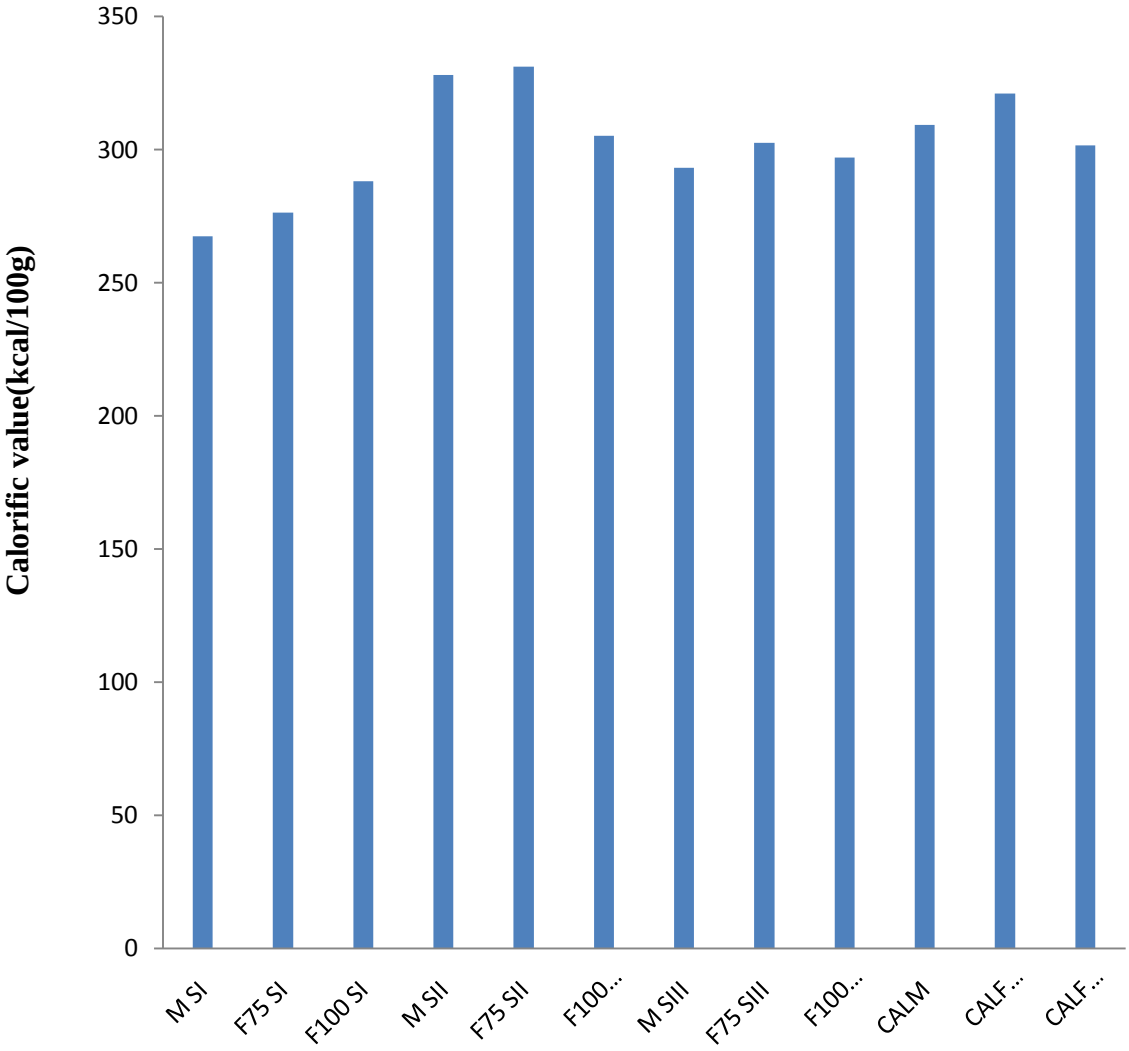


Figure 4.4: Proximate compositions of *Hibiscus sabdariffa*`s calyx (fruit)cultivated at different conditions



Proximate composition

Figure 4.5: Mean calorific value of*Hibiscus sabdariffa* at its growing stages andthe calyx cultivated at different conditions(SI,SII,SIII and the calyx)

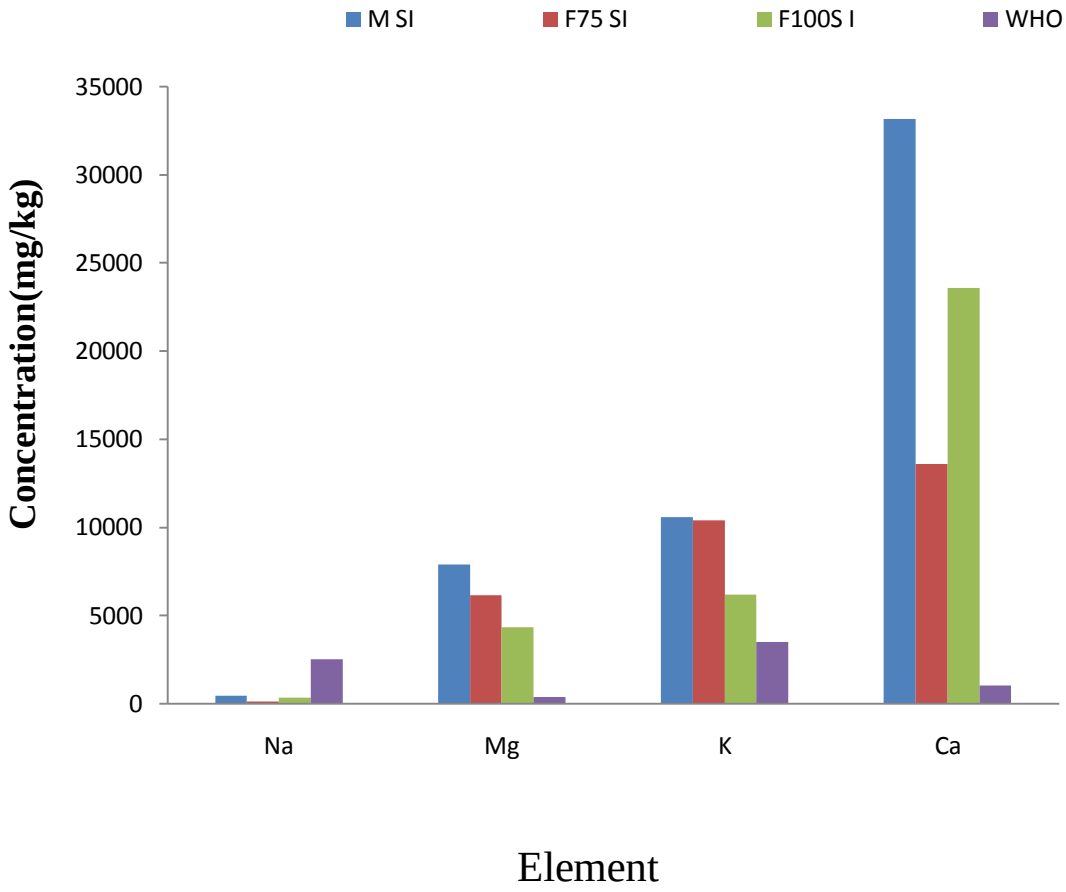


Figure 4.6**:** Mean concentration of essential elements of *Hibiscus sabdariffa* at a vegetative stage of growth cultivated at different conditions(SI)

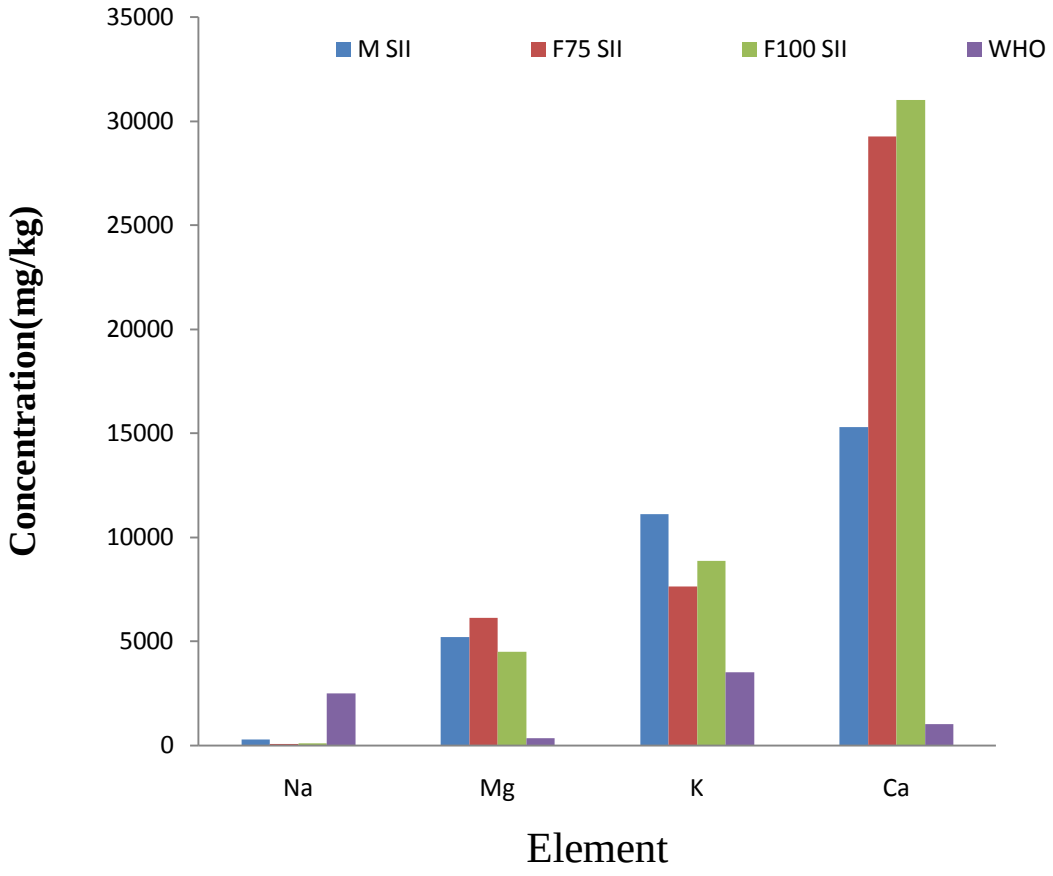


Figure 4.7: Mean concentration ofessential elements of *Hibiscus sabdariffa* atflowering stage of growthcultivated at different conditions(SII)

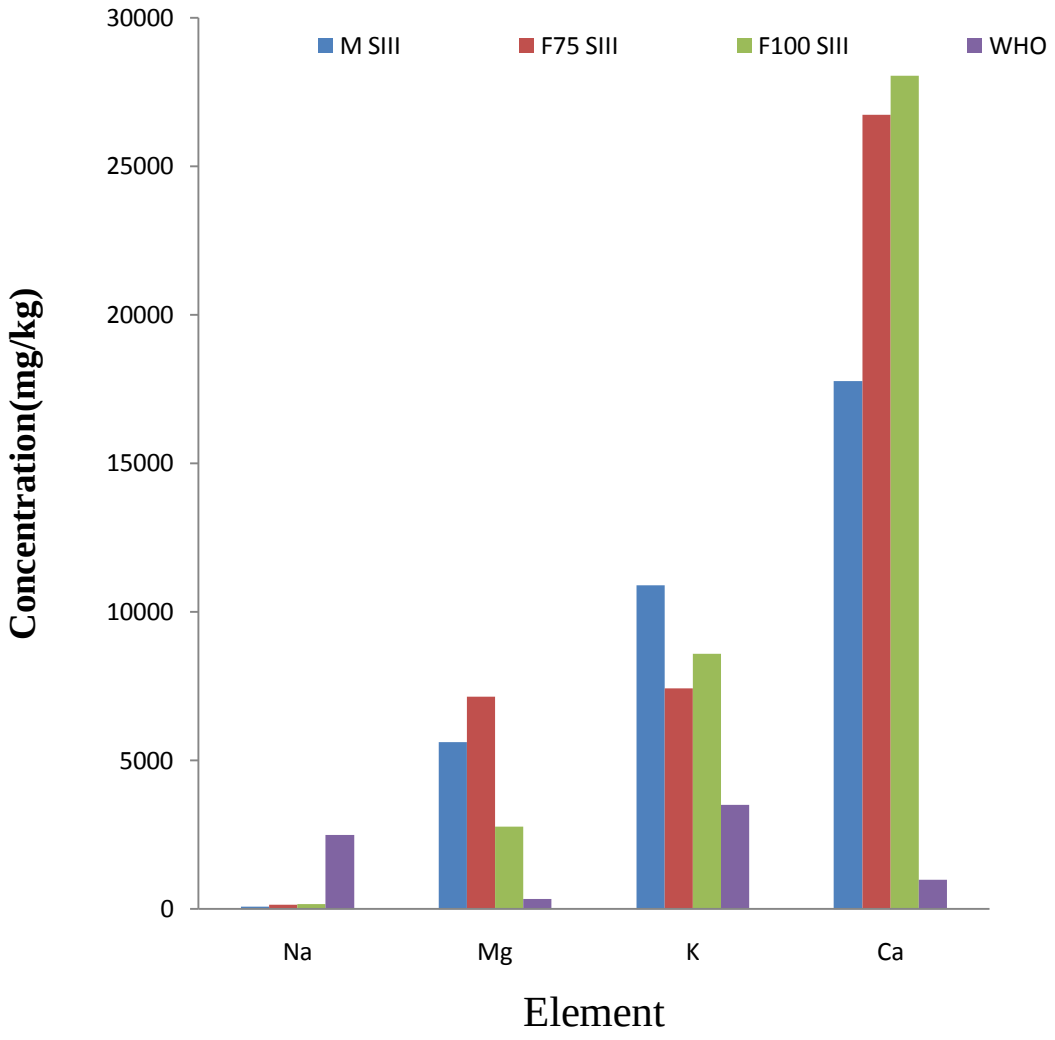


Figure 4.8: Mean concentration of essential elements of *Hibiscus sabdariffa* at maturity stage of growth cultivated at different conditions(SIII)

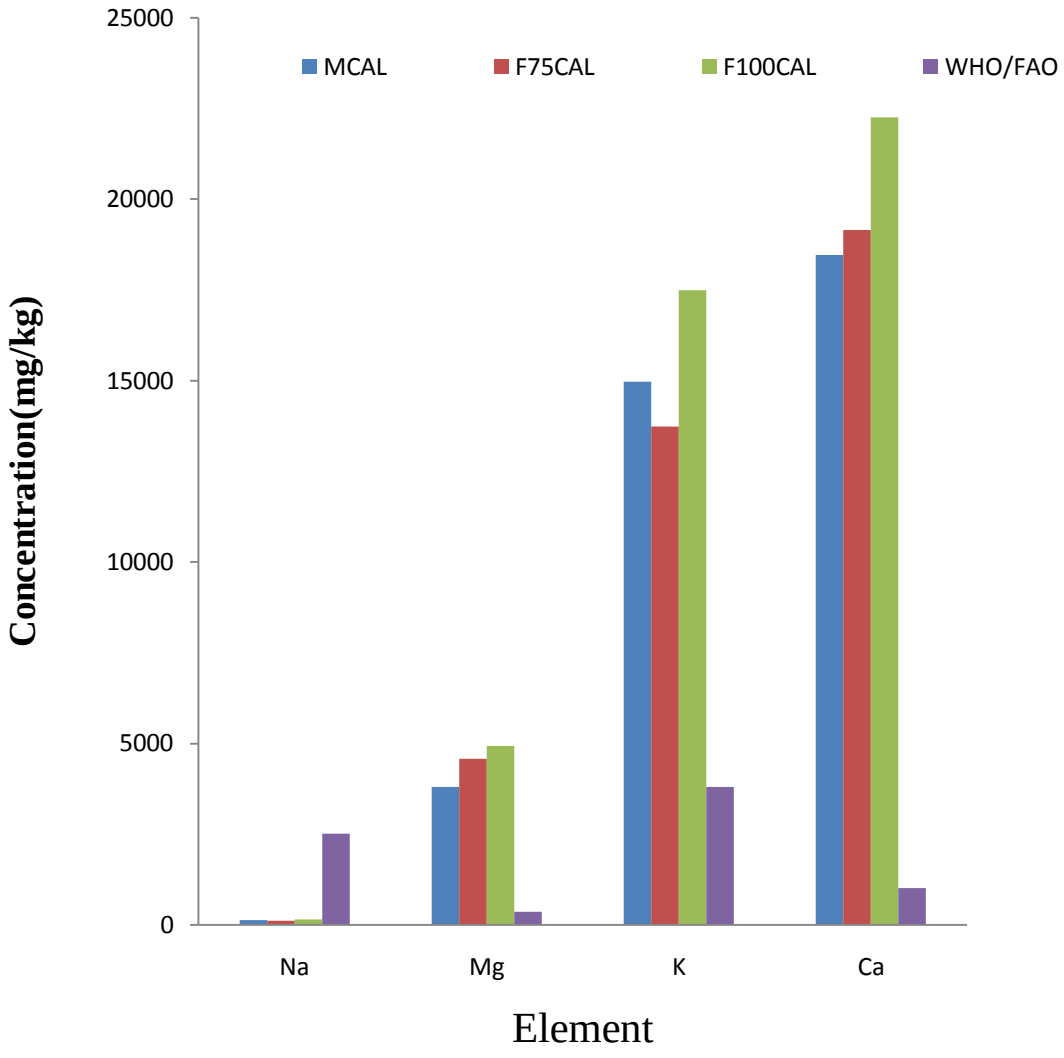


Figure 4.9: Mean concentration of essential elements of *Hibiscus sabdariffa`*scalyx (fruit)cultivated at different conditions

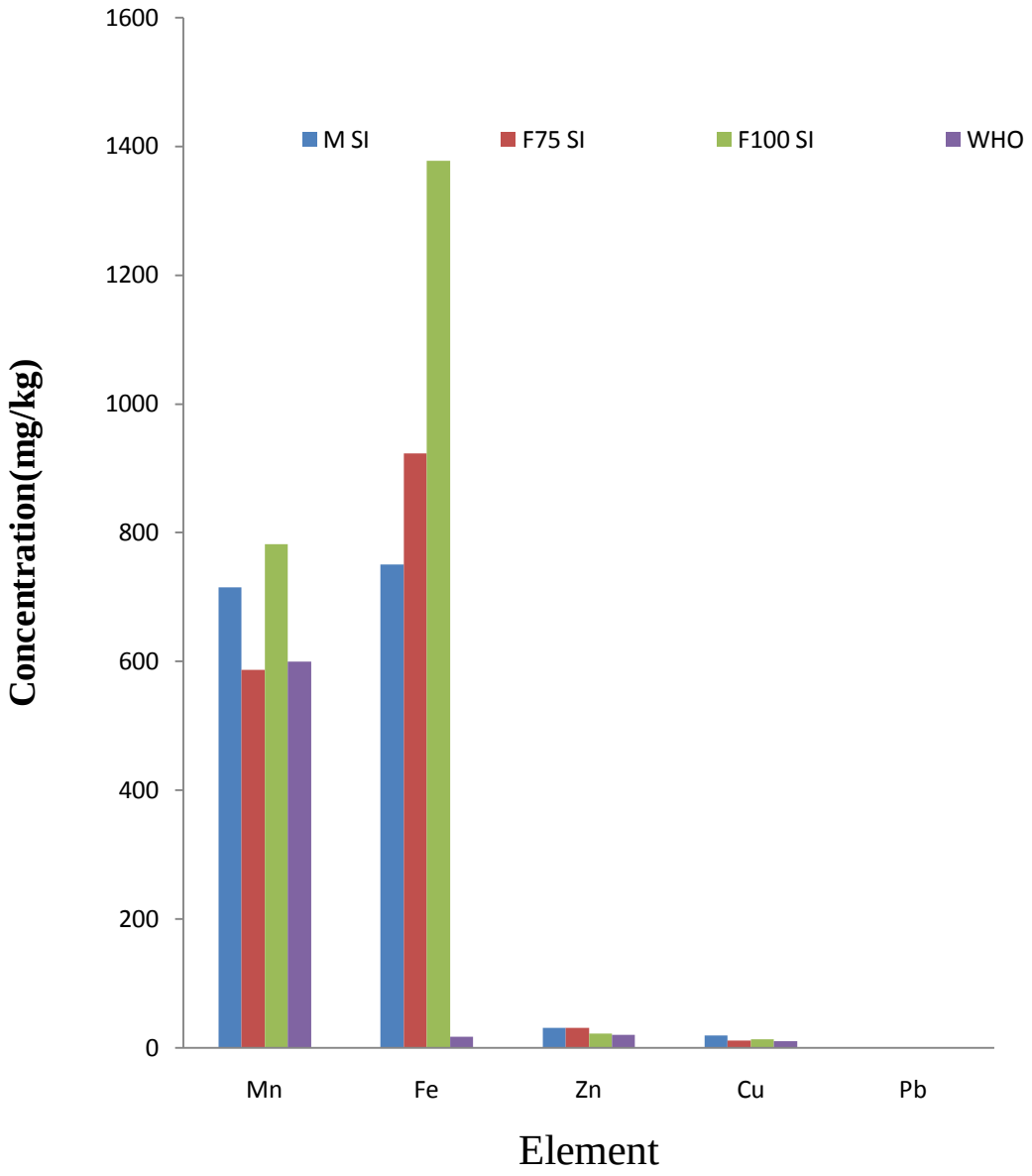


Figure 4.10: Mean concentration for trace elements of *Hibiscus sabdariff*a at a vegetative of stage of growth cultivated at different conditions(SI)

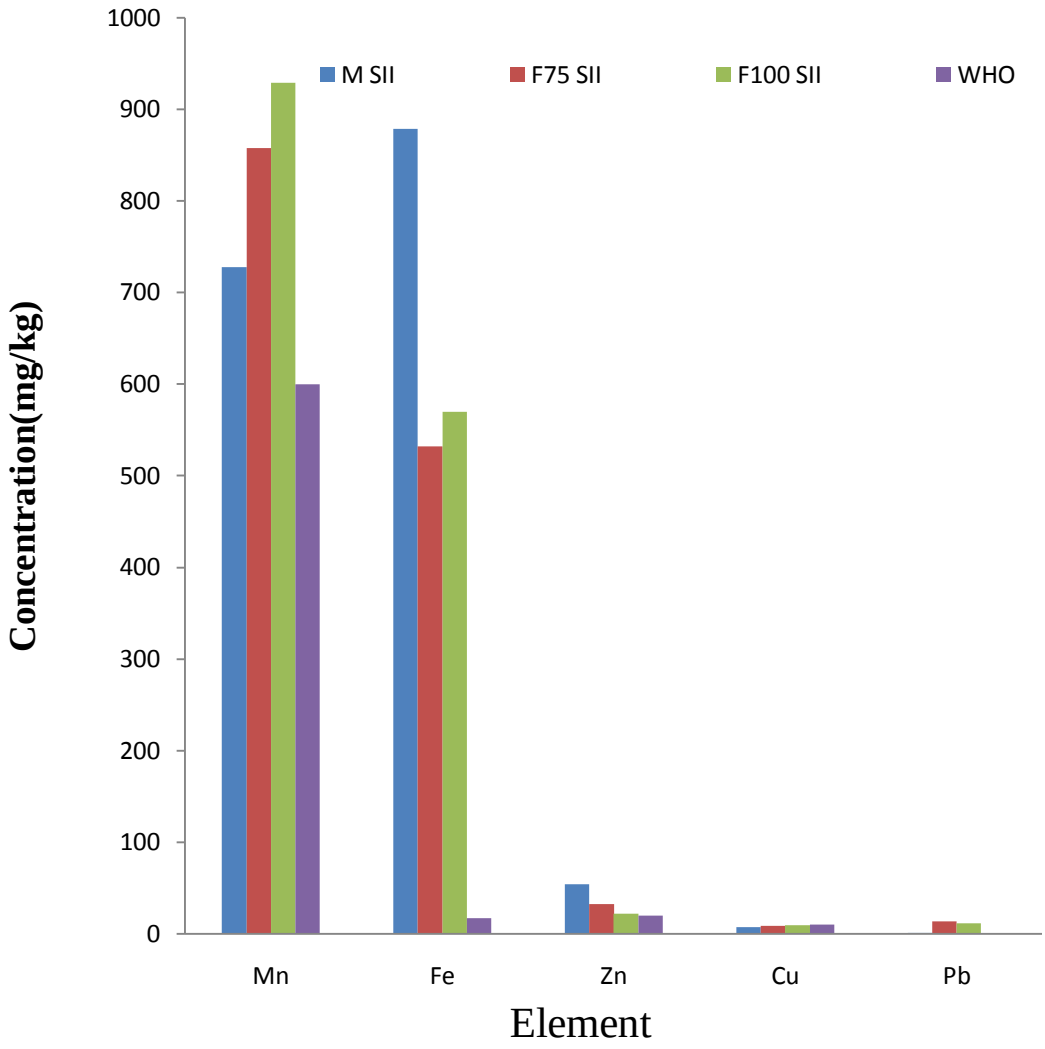


Figure 4.11: Mean concentration for trace elements *of Hibiscus sabdariffa* at flowering stage of growth cultivated at different conditions(SII)

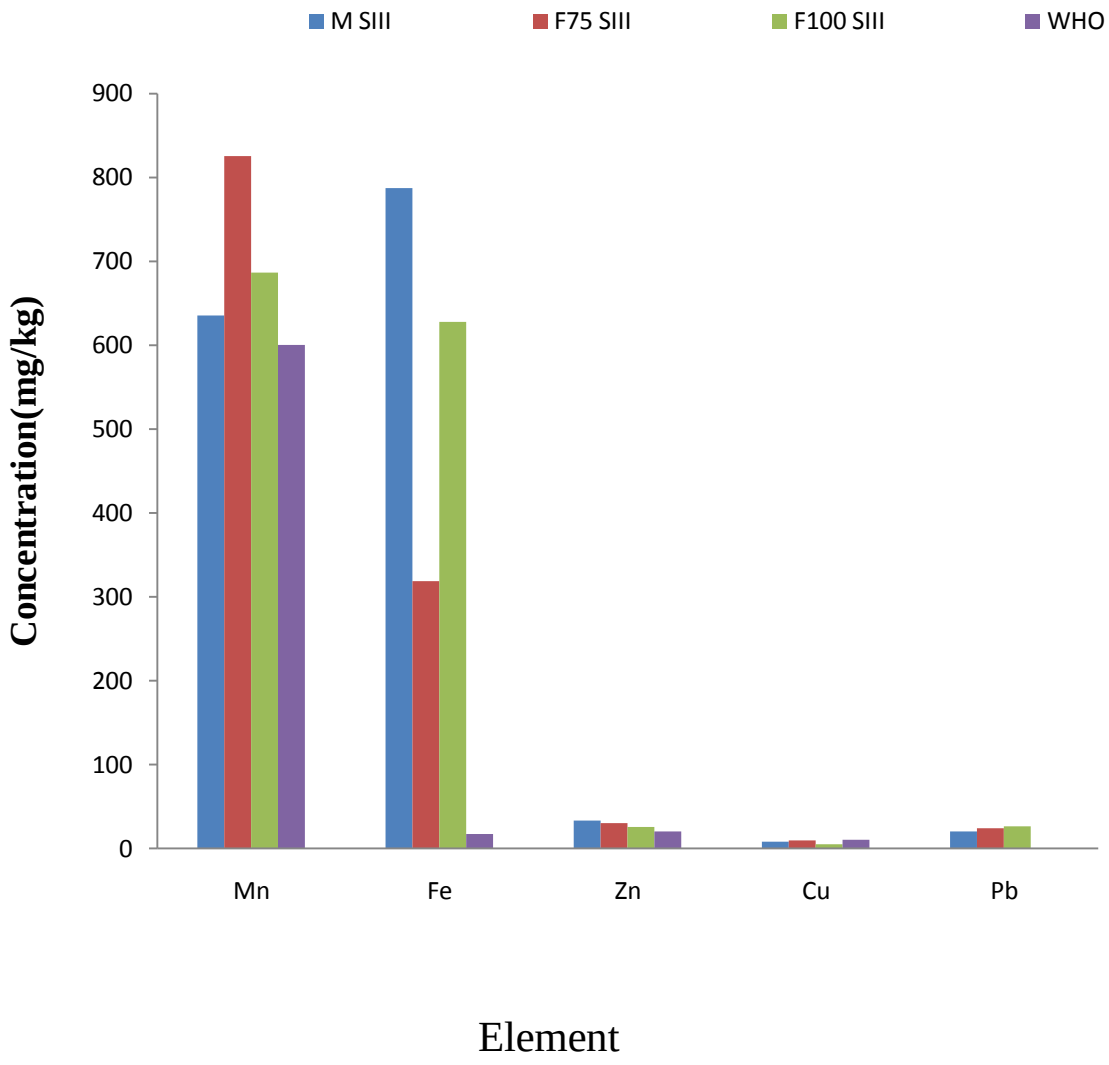


Figure 4.12: Mean concentration for trace elements of *Hibiscus sabdariffa* at maturity stage of growth cultivatedat different conditions(SIII)

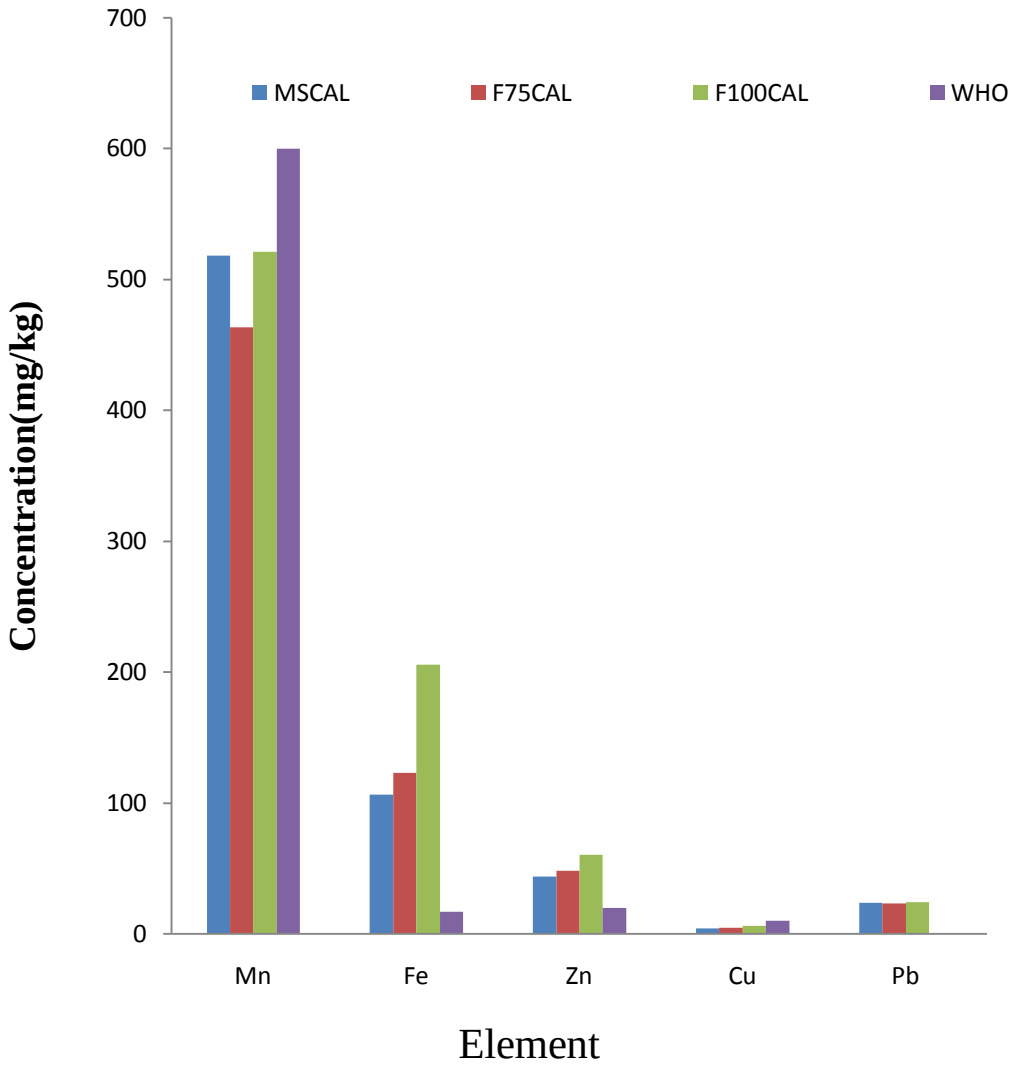
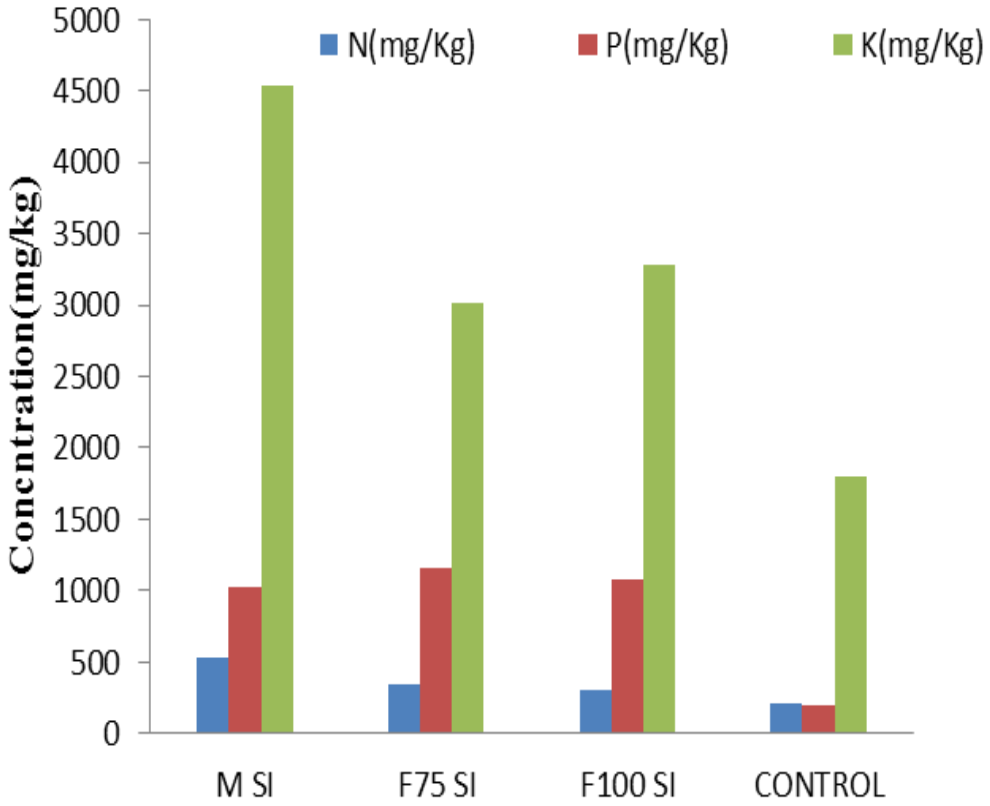
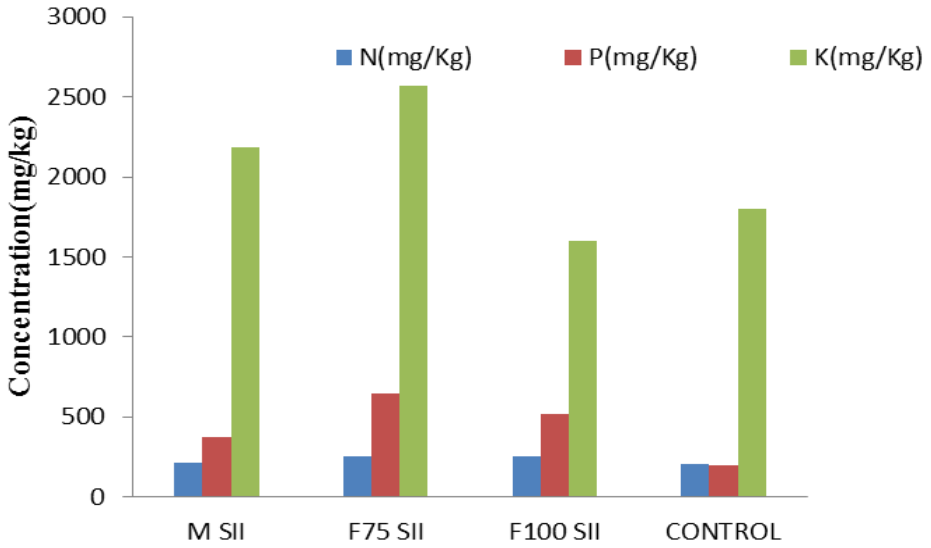


Figure 4.13: Mean concentration for trace elements of *Hibiscus sabdariffa`s* calyx (fruit) cultivated at different conditions



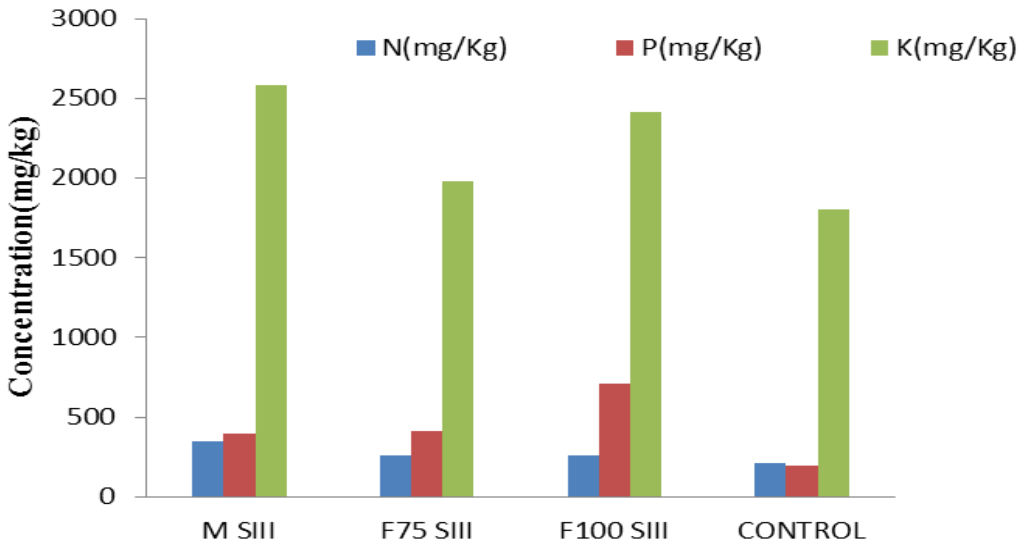
Soil sample

Figure 4.14: Mean concentration of macro elements of soil sampled at a vegetative stageof *Hibiscus sabdariffa*growthcultivated on different conditionsand control (SI)



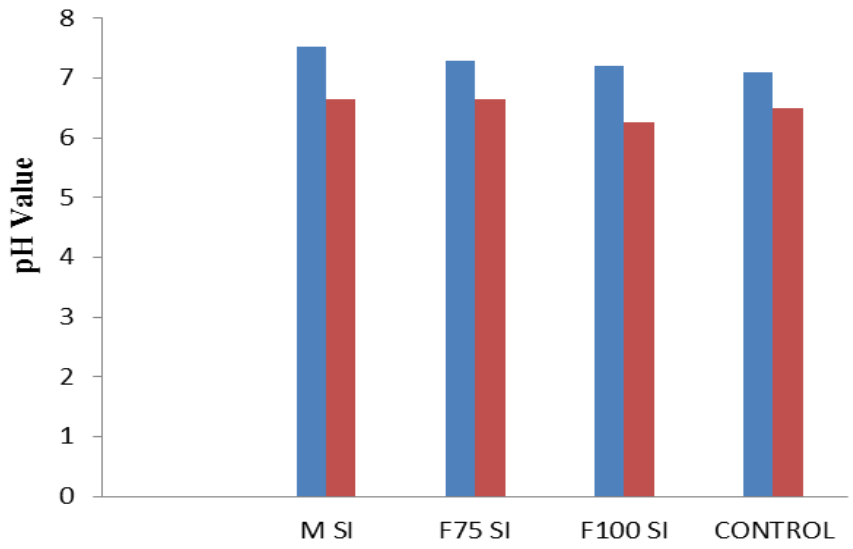
Soil sample

Figure 4.15: Mean concentration of macro elements of soil sampled at a flowering stage of *Hibiscus sabdariffa*growthcultivated on different conditionsand control (SII)



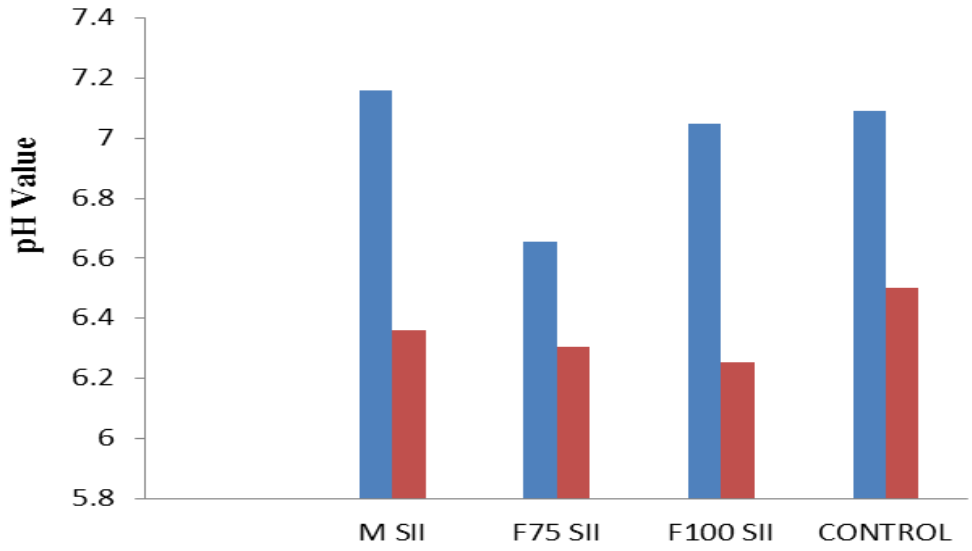
Soil sample

Figure 4.16: Mean concentration of macro elements of soil sampled at a maturity stage of *Hibiscus sabdariffa*growthcultivated on different conditionsand control (SIII)



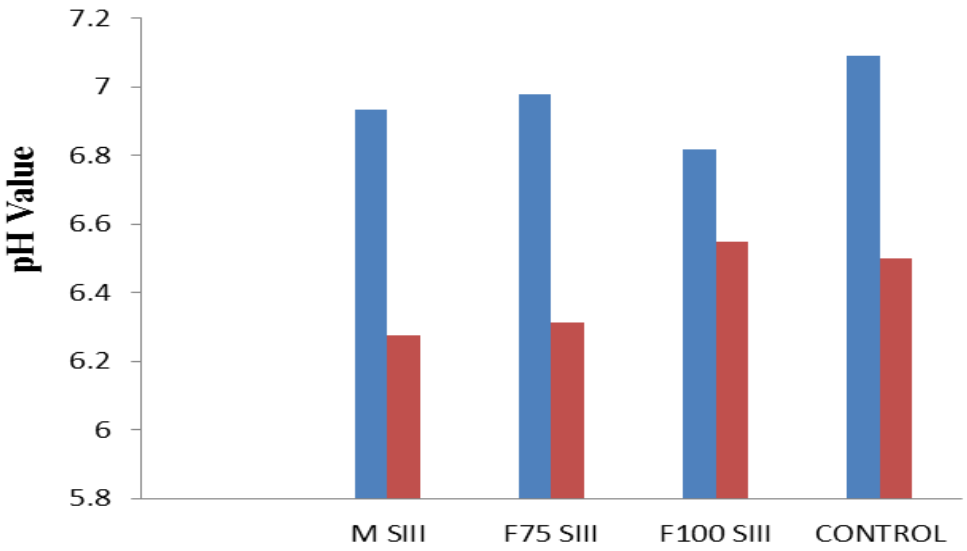
Soilsample

Figure 4.17: Mean of soil pH level in water and KCl medium for a vegetative stage of *Hibiscus sabdariffa*growthcultivated on different conditions and control (SI)



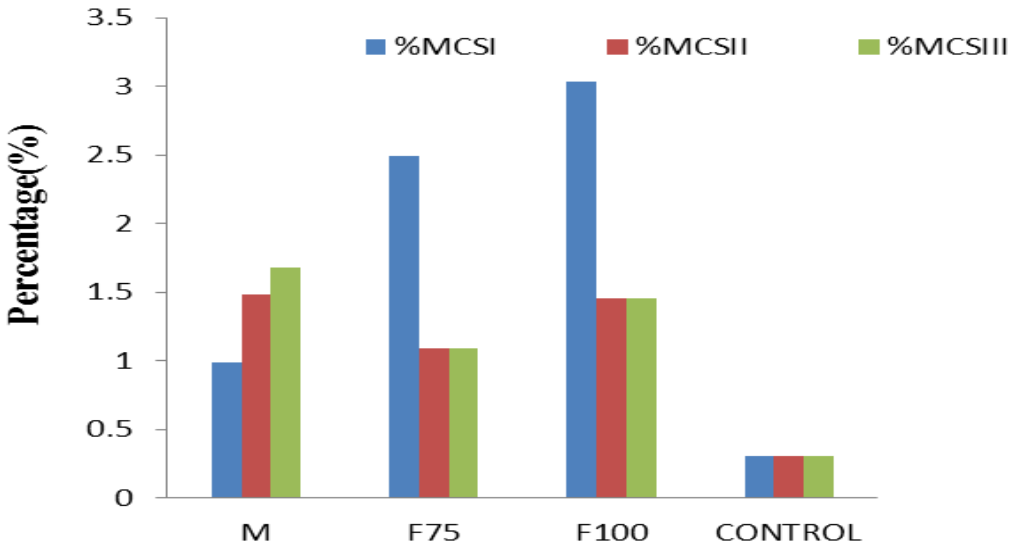
Soilsample

Figure 4.18: Mean of soil pH level in water and KCl medium for the flowering stage of *Hibiscus sabdariffa*growthcultivated on different conditionsand control(SII)



Soil sample

Figure 4.19: Mean pH level in water and KCl medium for a maturity stage of *Hibiscus sabdariffa*growthcultivated on different conditionsand control (SIII)



Soil sample

Figure 4. 20: Soil moisture contents atdifferent conditions and control across the stages of growth of *Hibiscus sabdariffa*(SI - SIII)

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Proximate Constituents at Growing Stages of *Hibiscus sabdariffa*

5.1.1 Moisture contents of *Hibiscus sabdariffa*

Moisture contents of the plant across the growing periodwerein the order of:stage III M> calyx F100 >stage II F100 >stage I F75. The moisture contents of foods or its processed products gives an indication of its freshness and shelf life, and high moisture content subjects food items to increase microbial spoilage, deterioration and short shelf life (Tressler *et al.*, 1980; Adepoju and Onasanya, 2008). Moreover, the estimated marginal means for the fertilizer levels on moisture contents,were in the following order of magnitude; M > F75 > F100 (Appendix VI).The maturity stage (stage III),had moisture contentthat increased with an increase in the concentration of fertilizer.Whereby, F100 treatment had the least moisture contents i.e (higher dry matter).The moisture contents decreased with an increase in mineral fertilizer concentration (Appendix VI). However, the moisture contents obtained in this work was within the tolerable limit of 10% when compared to other plant like, Cactus cladode reported with 18% (Sana, *et al*., 2017). The result of the statistical analysis, shows that the differences in the moisture contentsamongst the fertilizer treatments levels were insignificant ($p<0.05$).

However,the estimated marginal means with respect togrowing stage (Appendix VI),showedthat calyx >stage I>stage III >stage II, which means that fruit(calyx)washigherin moisture contents. The greater moisture levelof the fruit (calyx) was essential as, itgives the freshness to fruit (Warner, 2002). Stage II hadthe lowestmoisture contents, because,it was a flowering time where some of the biochemical reactions,involving moisture take place.

5.1.2 Ash contents of *Hibiscus sabdariffa*

The levels ofash in *Hibiscus sabdariffa* was in the following order:stage I F100>stage II M>stage III F100>calyx F100.The ash contents increased with concentration of fertilizer at stage III (Figure 4.3).On the other hand, the estimated marginal means for the growing period(Appendix VI)**,**revealed that, stage I had the highest ash contentsof 12.761%, followedby stage II(10.640 %), then stage III while calyx retained the least valueof 6.784% (Figure 4.1-4.4). Statistically, stage I ash contentswas significantly different from stages III and calyx, and also stage II shows level of significance to calyx at ($p<0.05$).Thetreatments estimated marginal meansindicated F100 treatment which has the highest ash content of 20.189%, followed byM, and the least ash content was found in F75 (9.482%).

Thefindings clearly showed that, ash content decreasedalong the growingstages (Figure 4.1- 4.4).The ash content was found to be maximum when high concentration of fertilizer (F100) was added (Figure 4.1- 4.4),which further indicate that to harvest the plant for the optimum minerals nutrient,F100 is a preferable fertilizerto be use except, when the plant is expected to be harvested at a flowering stage, where M fertilizer is preferable(Figure 4.2). The ash content, which is an index of mineral contents in biota, was found to be highinthe plant treated withF100 (20.189%). Ifon and Bassir, (1980) and Ladan *et al*., (1996), reportedthe ash content of 20.05%. Thevalue obtained in this studycompared favorably with that reported for *Ipomea batatas* (11.10%), *Vernonia colorate* (15.86%) and *Moringaoleifera* (15.09% DW) (Lockett *et al.*, 2000; Antia *et al*., 2006). It was however, higher than that of some Nigerian leafy vegetables such as *Ocimum gratissium* (18.00%DW) *and Hibiscus esculentus* (8.00% DW) (Akindahunsi and Salawu, 2005).

5.1.3 Crude fat of *Hibiscus sabdariffa*

Crude fat of the plant was inthe following order of magnitude:at SI F100>SIII F100>calyx F100> SII F100.The F100 fertilizer dominatedall stages and calyx in crude fat content.The estimated marginal mean for the stagesof growth revealed that, SI hadcrude fat content of 20.189% as maximum value followed by SIII>calyx> SII, as shown in Figure 4.1-4.4. The SIcrude fat content was statistically foundto be significant to stage II at ($p<0.05$). The estimated marginal means of fertilizer treatments were; F100(17.173%) > M > F75(11.754%) asshown in(Appendix VI). Therewas no significant difference among the treatments at ($p<0.05$). *Hibiscussabdariffa* was found to be an excellent source of lipid and F100 is a better fertilizer to cultivate the plant with purpose of harvesting for rich lipid content(vegetable oil). The least crude lipid content (9.259% DW) from F75 at stage IIis comparably higher to other reported values (8.3 - 27.0% DW) in some vegetables consumed in West Africa (Ifon and Bassir, 1980; Sena *et al*., 1998).However, it favorably differs from 4.20% reported for *Calchorus africanum* leaves and (1.85 to 8.71% DW) in some edible green leafy vegetables of Southern India and Nigeria (Agbo, 2004; Gupta *et al*., 2005).This implies that*Hibiscus sabdariffa* could be a good source of vegetables oil.

5.1.4 Crude proteins of *Hibiscus sabdariffa*

The crude protein contentvaries in the following order of magnitude; stage I M>stage IIM>stage IIIF100> calyx F100.This shows that M treatment predominantat stages I -II and F100 stage III and calyx.The crude protein content observeddecreases with the growth *Hibiscus sabdariffa*. The estimated marginal meansat stages of growth (Appendix VI), followed the following order: SI >SII >SIII >calyx.SI shows level of significant differenceto only stage II at $p<0.05$. On the other hand, the estimated marginal means for the treatments were: M(10.699%)> F100>F75 (7.609%). M was significantly different from F75 at $p<0.05$.This showed that M fertilizer was a better source of organic nitrogen for protein.Soils

with higher organic matter contents may immobilize more nitrogen (N) and reduce N loss to the environment. Otherwise, the depletion of available carbon will cause more rapid N turnover and losses (Compton and Boone, 2002).At this point, M fertilizer will bea better choice in the cultivation of *Hibiscus sabdariffa* for protein source.If maturity stage or calyx would be the targeted time to harvest the plant,then, F100 wasthe better option. According to Pearson (1976), plant foods that provide more than 12% of their calorific value from protein are considered to be good source of protein. SI toSIII and calyxhave all attained this requirement, and are especially good for a pregnant women who require 13 to 19g protein daily (Parisi and Wacker,1968), as such SI in ourstudy recorded mean value of 17.170%, and is equivalent to 47.2g,hence recommendedfor adults.Clearly*H. sabdariffa* can be considered as a good source of protein, which is an essential component needed for survival of animals and human being. Protein deficiency causes growth retardation, muscle wasting, oedema, abnormal swelling of the belly and collection of fluids in the body (Mounts, 2000).

5.1.5 Crude fiber of *Hibiscus sabdariffa*

The crude fibercontentfrom the growth stages, can be presented in the order of magnitude as; SII M> SI M> SIII F100> calyx F75,Fertilizer (M)treated sample superseded others in terms of the crude fiber content,which also decreases with increase in concentration of fertilizer (Figure 4.1 – 4.4). The estimated marginal means from the stage of plant growth were as follows;at SII>SI> calyxSIII had the least mean value of 10.649% (Appendix VI). The difference in crude fiber contentamongst the stages was significant between stages III and I at ($p<0.05$). This may be attributed to cell wall development with maturity.The treatmentsestimated marginal means, shows M had11.430% of crude fiber, which was the highest value, followed by F75 (11.369 %), and thenF100 (11.095%) the least.Manure was the best to grow and harvest the plant from stages I-II, but F100 and F75 are better for stage

III and calyx. This findingis in agreement with statement of Blair *et al*., (2006) and Edmeades, (2003) who stated that, an increment of mineral fertilizer concentration reduces the level of organic nitrogen which is the main component of amino acid (protein).Adequate intake of dietary fiber can lower the serum cholesterol level, risk of coronary heartdisease, hypertension, constipation, diabetes, colon and breast cancer (Ishida *et al.*, 2000; Rao and Newmark, 1998). The RDA of fiber for children, adults, pregnant and lactating mothers are 19 to 25, 21 to 38, 28 and 29g, respectively(Akubugwo *et al*., 2007).

5.1.6 Carbohydrate of *Hibiscus sabdariffa*

The level of carbohydrate contents in the *H. sabdariffa* plant growth stages as presented in (Figure 4.1- 4.4) showsthe order; calyx F75 > stage III F75> II F75 > I F75. Overall, the highest carbohydrate content was recordedin F75 treatment.This fertilizer (F75) could be consideredas the maximumfertilizer required for the optimum carbohydrate yield of *H. sabdariffa*, for its predominance on carbohydrate content across the stages and calyx.The estimated marginal means of the plant growing stages shows increase in the carbohydrate content at growing stages; calyx (59.554%) the highest, then stages III, II and stage I (38.850%), with lowest value. The estimated marginal mean in treatmentswas highestwithF75 (54.310%),then stages F100 (50.610 %), and M 49.742%. This further shows thatF75is a better fertilizer to be applied for the maximum carbohydrate sourcing.However, the difference in carbohydrate content amongst the treatments was found to be insignificant at ($p<0.05$). The carbohydrate content (61.725% DW) in the calyx, obtained from this work was higher than the levels of 20, 23.7 and 39.05% reported for the leavesof *Senna obtusfolia Amaranthus incurvatus* and*M.balsamina*, respectively(Faruq *et al*., 2002; Hassan and Umar, 2006). It is however lower than those reported for *Corchorus tridens* (75.0% DW) and sweet potatoes leave, 82.8% (Asibey-Berko and Tayie,1999). The recommended dietaryallowance

(RDA) values for children, adults,pregnant and lactating mothers are 130, 175 and210 g, respectively(Akubugwo *et al*., 2007). It implies that the higher value obtained here from F75 in the calyx(61.725%)will give 47.480gRDA for children and adult,which is equivalent to 35.271% for pregnant and lactating mothers.

5.1.7 Calorific value of *Hibiscus sabdariffa*

The calorific value across the growing period wasin the order of magnitude; stage II F75> calyx F75> stage III F75 > stage I F100 (Figure 4.1- 4.4). The estimated marginal meansfor the growing stages (Appendix VI) indicated that, the calorific value fromstage II had the highest mean value, followed by calyx, then stage III and stage I(277.373 kcal). The treatments estimated marginal means revealed that F75 had the highest calorific valueof (307.833 kcal)then M (299.497 kcal) and F100 of298.112 kcal. The statistical test hadshownaninsignificant difference in energy content amongst the treatmentsat ($p<0.05$).Thefinding of this work also revealed that, F75 fertilizer is the best fertilizer for cultivation of *H. sabdariffa* plant across the stages of growth and calyx for energy content except whenthe plant is to be cultivatedand harvestedat a vegetative stage where F100 will be the preferred fertilizer. The meancalorific value (331.197 kcal/100g DW) obtained from stageSIIof *Hibiscus sabdariffa* growth, wasmaximum and higher as compared to other findings (268.92 kcal/100 g DW) in *A. hybridus* leaves, 248.8 to 307.1 Kcal/100 g DW reported in some Nigerian vegetables (Akubugo *et al*., 2007;Isong *et al.,* 1999; Antia *et al*., 2006). Asibey–Berko and Tayie (1999) also reported comparable energy content in some Ghanaian green leafy vegetables.

5.1.8 Oxalate contents of *Hibiscus sabdariffa*

The trend of oxalate contents in the analysedsamplesof *H. sabdariffa* was: stage II M> II F100> I M>calyx F100. The levels of oxalate contentswas found to be highestat stageII

(flowering stage) and lowest from stage III (maturity stage), except for F75 fertilizer treated samples. The oxalate content decreases at stage I and relativelyincreases in the calyx withincreasing fertilizer concentrations. The estimated marginal means revealedthat,stage II had the minimum mean value of 7.426%, followed by calyx, then stage I, andstage III with the highest mean value of 11.3187%(Appendix VI). The oxalate content was significantly differentamongst the other stages and the calyx ($p<0.05$).The estimated marginal means fortreatments were in the order of magnitude; where M had the highestmean value of 9.800% followed by F100, then F75, 8.720%.Thismaybe attributedto the available organic carbon in M fertilizer which is the main component of an oxalate. The level of oxalate contents was insignificant at ($p<0.05$) for other treatments.The level of oxalate content in thegreen and variegated cultivars of sorrel were:14.7 and 24.4g/100g (Tuazon andSavage, 2013), which were higher than the values obtained in this studied plant. When comparingthe levels of oxalate content obtainedin this work to that of WHOtolerable limit (256mg/100g),the raw sample of *H. sabdariffa*had higher oxalate content.Ruan *etal.,* (2013), reported that boiling in water reduced the level of the anti-nutrients oftaros plant by at least 47%.Hence, it is recommended to boil this plant before used.Oxalates has bio-pesticide properties in bee-keeping as its advantage, it can bind to calcium present in food thereby rendering calcium unavailablefor normal physiological and biochemical role, such as maintenance of strong bones, teeth, cofactor in enzymatic reaction, nerve impulse, transmission and as clotting factor in the blood(Inuwa *et a*l., 2011).

5.1.9 Tannin contents of *Hibiscus sabdariffa*

The tannin contentsatthe growing stages, increasedfrom stages I-III.In stages I to II, F75 had the maximum valueof tannin content (2.857%)while, stage III and calyx were found to be higherin tannin with M and F100 (5.230%)fertilizers.Theestimated marginal means

across the stages ofgrowthshows an increasein tannin content with maturity of the plant.The calyxhadthe highest tannin level (4.042%), followed by stages III, II and I (2.726%).The difference in tannin levels amongst the growing stages became significant at ($p<0.05$). However, the treatmentsestimated marginal means shows, F75 with highest mean value(3.350%), then F100, and M treatment with least value 2.257% (Appendix VI). Theconcentration of mineral fertilizer reduced tannin content in the calyx (Figure 4.3) and increase the tannin at stage I (Figure 4.1) of plant growth.Lower value in tannin content was recorded at stage I. However, the increase in tannin content with maturity of the *Hibiscus sabdariffa*plant was an indication that vegetative stage willbe a better time for harvesting the plant. Comparably,the levels of tannin was found to be higher than the value obtained for plantain root (*Musa paradisiaca*), 0.079 mg/g, (Ayoola, 2011), and lower than those obtained by Adeolu and Anesi, (2010), (115 mg/100g) from plantain.The tannin contentobtained here wasfound to be within the safer level when compared to detrimental level of 76-90g/kg (Aletor, 1995).Additionally, the lowest concentration of tannin was observedin M treatment which clearly indicates that it was the safer fertilizer.

5.2 Minerals Components of *Hibiscus sabdariffa*at its Growing Stages

The minerals components of *H. sabdariffa* are presented in Figures (4.6 to 4.14).

5.2.1 Sodium (Na) contents of *Hibiscus sabdariffa*

Sodium contents were decreasedfrom stage I to III.The mean Na content followed the order; SIM (448.903 mg/kg)>SII M>SIII F100> calyxF100 (142.433mg/kg). At the maturitystage(SIII), Na concentration increased with an increase in theconcentration of fertilizer. However, the estimated marginal means(Appendix IX)revealedthat SIwas highest in Na(300.896mg/kg).M treatment had ahigher mean value of Na (232.460mg/kg), followed

by F100 and then F75 (106.524mg/kg) the least. The Na concentration ofM treatment was significantly different from other treatmentlevels at ($p<0.05$).

In comparison to other findings, *H.sabdariffa* calyxhad 72.10mg/100g of Na (Wahid *etal.,* 2012), 3.20, 3.40 and 1.60mg/g reported for the three ecotypes E3, E7 and E9 varieties of calyx(Atta *et al.,* 2013). The concentration of Na (448.903mg/kg) recorded in this studyis higher when compared to 7.43mg/100g reported for leaves of Amarantush.(Akubugwo *et al.,* 2007).The maximum permissiblelimit for sodium is 2500mg/day (WHO,1996). The Na contents obtainedin this study werefound to be within the WHO permissible limit, in which M fertilizer had the higher Na content from stage I-II and could be the best fertilizer of growing the plant for harvest at this stages, where, F100 wasthe highest in Na concentration for SIII and calyx of the plant growth,which possibly which might be the excellentfertilizer for dietary source of sodium if plant is to be harvested at SIII orthe calyx. Sodium and potassium are essential elements for fluid balance and nerve transmission. The Na content decreases with *H.sabdariffa* development as observed from this work,in which similar finding was reported by (Atta, *et al,* 2010).

5.2.2 Potassium (K) contents of Hibiscus sabdariffa

The treatments estimated marginal means of K provided a greater value from M fertilizer, next to it was F75, then F100.This indicates that there was a decreased in K content with an increased mineral fertilizer level (Appendix IX). There was no significant difference amongst the treatments at ($p<0.05$). However, the estimated marginal meansof K forgrowing stages, revealed higherconcentrationof Kin the calyx (15391.751mg/kg), followed bySII, then SI and SIII (8979.498mg/kg).TheK concentrationreported from otherplant like,*Amarantus. h,*54.20 mg/100g.(Akubugwo *et al.,* 2007) and calyx 101.5 mg/100g(Wahid *etal.,* 2012) were all below the concentration recorded in this study. Similarly, the concentrations of 1322,419,868,893,2292,2847,3220 and 5540 mg/100grecorded in *Launaea.t, Ceiba.p,*

Manniophyton.f,Talinium.t,Corchorus,Amarantus.i,Manihot.u and Hibiscus.s(Dickson *et al.*, 2012), and were below the Na concentration recorded in this study with few exceptions.Consequently, to harvest the plant for the purpose of K supplementation M fertilizeris recommended.The average adult requirement for potassium is 4700mg/day (Krauss and Eckel, 2000), going by this requirement, *Hibiscus sabdariffa*will be a good source for K throughout the stagesof its growth and calyx. The availableK contentsrecorded from this vegetable plant would further make *Hibiscus sabdariffa* valuable as a raw material for indigenous soap industries and also in the treatment of soils acidity.

5.2.3 Magnesium (Mg) contents of *Hibiscus sabdariffa*

The Mg mean value obtained atthe treatments levels across the growing stages recoded with highvalue for M fertilizer atSI (7881.915 mg/kg), followed by F75 SIII, then F75 SII, and the least value recorded in the calyxF100 (4927.246mg/kg).Mg content was found to decrease with increased in fertilizer levels at vegetative stageof plant growth (SI)(Appendix IX) and Mg concentration found increased with increase of fertilizer concentration (Figure 4.9). On the other hand, the estimated marginal means for the treatments (Appendix IX) shows F75 fertilizer had the highest Mg content (5997.851mg/Kg), then M, and F100 treatment the least (4122.833mg/Kg). Statistically, treatment M and F75 were significantly different from F100 in Mg concentration at ($p<0.05$),which implies that F75 and M aregood source of Mg especially at the flowering and maturity period (SII and SIII), because, most of the elements concentration decreases as the plant maturity time approaches.The F75 if used for crop growing will enhance Mg concentrationat the time when element perishes (i.e during flowering stage). M and F100 fertilizer could be selected for early harvest and calyx produce relatively. The estimated marginal means ofthe growing stagesrevealed that, SI hadmaximumMg contents (6119.044mg/Kg).The differencein Mg

content amongst the stageswas significant at ($p<0.05$). As compared to Dickson *et al.*, (2012) findings, Mg contents fromsome plants like, *Launaea.t, Ceiba.p, Manniophyton.f,Talinium.t, Corchorus,Amarantus.i,Manihot.u and Hibiscus.s*were: 97.7, 35.5, 96.7, 30.2, 35.0, 106.1, 43.1 and 173.9 mg/100g,respectively, here *Hibiscus.s*had the highest value of Mg, which was still below the concentrations recorded in this study (Appendix IX).Mg content in *Amarantus.u* leaves was found to be 231.22 mg /100 g (Akubugwo *et al.*, 2007),still was far below7881.915mg/Kg from M fertilizer at SI (Figure 4.6).Magnesium is an essential element that plays a key role for the maintenance of normal muscles, nerves function and strong bones. The recommended daily intake value for Mg is 400mg, FDA, USA. However, as explained by Institute of Medicine, (1999), vegetable plants which provide between 10-19% of the magnesium daily value are described as good source.However, those that provide 5% or less are poor.*Hibiscus sabdariffa*as studiedin this work will be a good source of Mg.

5.2.4 Calcium (Ca) contents of *Hibiscus sabdariffa*

Cawas found to increase with an increased fertilizer level across the stages investigated except at SI. The values of Ca content continuously increase with concentration of fertilizer especially atflowering and maturity stages and then calyx (Figure 4.7 – 4.8, and 4.9).The estimated marginal means ofgrowing stages, revealed a higher value obtained from stage II (25180.950 mg/kg)as comparedto stage III(Appendix IX). The treatments estimated marginal means therefore indicates that F100had a maximum value of (26223.289 mg/kg) of Ca, followed by F75, then M treatment (21178.656mg/Kg). This indicated that Ca content increaseswith an increase in mineral fertilizer level to the soil. The differences in Ca level from the growing stages and treatmentslevels were found statistically insignificant($p<0.05$). Ca content was found to decrease bythe growth and development of the plant from SI and II as the concentration improved from SIII and calyx with M fertilizer.The F100 fertilizer was

found to be the best to be recommendedfor growing the plantfor Ca supplement.The Ca concentrations recorded in this work were greater (19952.325 to 25180.026 mg/kg)when compared to other reports of Akubugwo *et al.*, (2007) and Wahid *et al*, (2012) on plants *Amarantus u.* and *Hibiscus sabdariffa calyx*; were recorded (44.15 and 14.8mg/100). The permissible limit for Ca is 1000mg/day (WHO, 1996). It is obviousthat, some cereals flours commonly consumed are deficient in calcium, for example, pearl millet, the fortification of flours with Roselle leaves or calyx flour might improve their dietary properties as suggested by El Adawy and Khalil (1994).

5.2.5 Manganese (Mn) contents of *Hibiscus sabdariffa*

The Mn concentration observed increases with an increase in the fertilizer concentration at flowering stage.The estimated marginal means (Appendix IX)shows the same pattern with calciumcontents as stage II recorded maximumvalue of 837.855mg/kg, followed by stage III, thenstage I, and the calyx with least value (500.890mg/Kg). The difference in Mncontent among the stageswas significant at ($p<0.05$). In terms of the estimated marginal means for the treatments, F100 possess the maximum value of Mn 729.534 mg/kg, followed by F75, and M has the minimum countof 648.981 mg/kg. However, the differencein Mn contents amongstthe treatments was insignificant at ($p<0.05$).Mn concentrationsincreased with an increase of fertilizer concentration from SI to II in*Hibiscussabdariffa's*growthwhich was found to be in accord with the report of Atta *et al.*, (2010)findings from three ecotypes. However, at maturity stage (SIII), Mn contentmaintainedits concentrationwith F75 fertilizer.Wahid *et al.*, (2012), reported 10.8 mg/100g Mn content from calyx which was below our findings, the permissible limit requirement for healthy living(600mg/kg)and alsotolerable daily intake 12mg/day (FAO/WHO, 2001). This clearlyshows that Mnis trapped maximallyduring mineral fertilizer treatment especially from SI - III. Manganese is crucial to human physiology.It is also

significant for haemoglobin formation.The quantity of manganese requirement in the body is small as compared to iron. However, *Hibiscus sabdariffa*plant could be a good supplement of Mn especially at floweringstagewhere the nutrients content replenish.

5.2.6 Iron (Fe) contents of *Hibiscus sabdariffa*

The iron contents across the growing stagesdecreasewith time as stage Iwith F100>stage II M>stage III M > calyx F100. The concentrationof Fe was higher with F100 treatment except in stage II, Fe concentration increased with fertilizer concentration in the calyx (Figure 4.10),hence F100 could be abetter fertilizer for calyx production.However, an estimated marginal means for Fecontentfrom growing stages, shows that SI>SIII>SII>calyx (144.852mg/kg). Statistically, the differencein Fe contentwas significant amongthe stages I - III and calyx ($p<0.05$).Moreover, the treatments estimated marginal means shows highest value of Fe in F100 (695.102 mg/kg), followed by M fertilizer and then, where the lowest concentrations of 474.038mg/kg was recorded. Thus,a decrease in Fe contents with increased mineral fertilizer concentration was observed. This is because, addition oforganic manure increase the water holding capacity of the soil, also enhance the microbial decomposition of organic matter and providing the excess moisture contents in the soil for easy nutrients assimilation by the plant.

Mineral fertilizer as observed in this finding reduced the moisture content level drastically which restricted the mobility and availability of nutrients. The reduction ofFe content from, SI to II with F100 treatment is in conformity with the finding of Atta*et al.*, (2010), who observeda decreasedin Fe from SI-II on plant ecotypes.The concentration of 8.5mg/100g Fe reportedin calyx byWahid *et al.*, (2012), in*Amarantus.U* leaves, 13.58 mg/100 g by Akubigwo *et al.*, (2007), in*I batatas* (16.00mg/100 g) by Antia *et al.*, (2006), comparedfavorably with findings in this study.The Fe contents reported werebelow those obtainedhere except,calyx treated with M and F75 fertilizers(Table 4.6).Theplant (*H.*

sabdariffa)meet up the required dietary value (DV) of 18mg/day as recommended for iron (Stoltzfus and Dreyfuss, 2012). Food that can provide 10-19% Fe, is a good source of Fe.The least value obtained in calyx in this study is enough to provide 76.06% Fe, which is higher than 72% obtained by Dickson *et al.*, (2012). Fe is very useful in the prevention of anemia and other related diseases.This plant can serveas supplement for iron deficiency, the most common form of malnutrition in the world.This work clearly showed that, the *H. sabdariffa* plant can be a good source of non haeme iron if bio-available, as iron of plant origin is poorly absorbed in the body (Roth and Townsend, 2003).

5.2.7 Zinc (Zn) content of *Hibiscus sabdariffa*

Znconcentration washighestin M fertilizer treated samplesexcept the calyx. The concentration of Zn contents decreased with an increase of fertilizer level at stages of growth (Figure 4.10- 4.12).However, Zn concentration increased in the calyx with an increase of mineral fertilizer concentration (Figure 4.13).The estimated marginal means forZn from the stages of growthshowsthat; calyxwas higherin Zn (50.621mg/kg), followed bySII, thenSIII, while, stage I offeredthe leastZn content (27.558 mg/kg). On the other hand,theestimated marginal means for the treatments levels revealed that: M fertilizer had the greater value of 40.416mg/kg, then F75, and F100 (32.299mg/kg)as presented in Appendix IX.Thiswork agrees very well with the work of Atta*et al.*, (2010),who reported a decrease in Zn content from stage I to II of *H. sabdariffa* growth. However, Zn content increasedin the other treatments (Appendix IX).Thisisadditional information to the work of Atta *et al.*, (2010)who have used a single treatment level of fertilizer instead. It was basically observed that Zn concentration decreased with mineral fertilizers at each stage of growth (SI-III) except calyx, but, Fe increased with M fertilizer at these stages except in the calyx (Figure 4.10-13), which further indicate the proficiency in using M fertilizer as choice for these elements. Moreover,

the recommended dietary value (RDV) of zinc for infant and adult are between 3-11mg and 50mg/kg respectively which is the recommended permissible tolerable weekly intake (WHO, 1993).*Hibiscus sabdariffa*plant can therefore be a good supplement of Zn, because, even the least value of (21.376mg) reported was equivalent to 19.4% (RDV). Znis an essential element of living organism for its involvement in a large number of enzyme and stabilizing molecular structures of membrane (WHO, 1996). Zincalso participates in the synthesis and metabolism of lipids, carbohydrates and proteins. It also has the capability to interfere with metabolism of other metals such as Cu. Hambidge, (1987), noted that deficiency of Zn could lead to stunted growth and delay sexual maturation. Thus, from our finding,*Hibiscussabdariffa* would be a good source for dietary Zn,while, to harness its abundance, manure fertilizer is the best option inits cultivation.

5.2.9 Copper (Cu) contents of *Hibiscus sabdariffa*

Cu contentsobserved across the growing stages,showsthat stage I hadthe maximum concentration with M(18.571mg/kg), followed by stage III F75,stage II F100 and then calyx also with F100 (5.738mg/kg). TheCu concentration increased at stage II and calyx forincreased infertilizer level (Figure 4.11 and 4.13). However, the estimated marginal means shows that, concentration of Cu decrease with*Hibiscus sabdariffa* plant growth, stage I hada maximum value of 14.073mg/kg, while calyxhad the least value of (4.747 mg/kg). The difference in Cu content that exists amongst the stages were statistically significant from stage I to other stages and calyx at ($p<0.05$). The treatments estimated marginal means, weighed M fertilizer ahead ofother treatments in concentration with (9.572mg/kg)value, followed by F100, and F75 hadthe lowestvalue (8.337mg/g).The difference among the treatment wasinsignificantat ($p<0.05$). This clearly revealed that, poultrymanure (M) is a better fertilizerfor the cultivationof*H. Sabdariffa*for the conservation of available copper,

especiallyatvegetative stage.Though, Cuconcentration decrease with growth of*H. sabdariffa*,the result compensated thatfor harvest, at flowering stage (SII) or for calyx.It required additional concentration of mineral fertilizerfor the maximumyield of Cu contents. The Cu content obtained from this work was above 73, 49, 67µg forthe three ecotype calyces reported byAtta*etal.*,(2013).JECFA suggested that Cu safe limit was 40 mg/kg for adult (FAO/WHO, 2001).The concentrationsof Cu reported in this study were within the permissible limit.Cu is essential to human life as metallo-proteins and function as enzymes.However, critical doses lead to health risk such as anemia, diabetes, inflammation, kidney and liver dysfunction and vitamin C deficiency (Lokeshappa *et al*., 2012).This justifiably qualified that the fertilizerstreatments used for this work are safe in the production of *H. sabdariffa* plant for Cu supplementation.

5.3 Soil Physicochemical Parameters

Figure 4.14 - 4.20, shows thesoil physicochemical parameters andAppendix XII, presents the estimated marginal means of parameters (at growing stages and treatments).

5.3.1 Nitrogen (N) contents of the soil

The nitrogencontent across the growing stages washighest at stage I with M treatment (536.667mg/Kg).M fertilizer gives higher nitrogen value at both stages I and II.The estimated marginal means forthe growing stages was highestat stage I (396.667mg/kg), and continuously decrease from stages III to II (241.111mg/kg). The control had the least value of nitrogen (210.000mg/kg). In terms of the treatments estimated marginal means, M treatment had the maximum value of 326.667mg/kgfornitrogen, then followed F75, and F100 had the lowest concentration of 256.667 mg/kg. The differencesin nitrogen content was significant at ($p<0.05$)across the growing stages.Thisdifference was statistically insignificant among the treatments at ($p<0.05$).It can be inferred that,the available nitrogen content decreases with

growth of the plant and also with an increment in concentration of mineral fertilizer. Poultry manure is an excellent source of organic soil nutrients, as it contains high nitrogen, phosphorous, potassium and other essential nutrients (Ibrahim *et al*., 2014).Organic manure application is considered to be a consistent method for maintaining soil fertility over the long-term (Blair *et al*., 2006: Edmeades, 2003), and demonstrated that nutrients from organic manure was important nitrogen source for crop growth and excessive mineral fertilizer application was wasteful without considering nutrient from organic manure. This is evident fromthe higher dry matterobserved in F100 treatment for *H. sabdariffa* plant growth toward a maturity time.This clearly indicates that manure was the best fertilizer for improving the available soil nitrogen followed by F75 in cultivation of *Hibiscus sabdariffa*plant.

5.3.2 Phosphorus (P) contents

The level of soilphosphorus presented in (Figure 4.14 to 4.16), revealedthat the maximum meanvalue was recorded in F75 at stage I(1153.583mg/kg). Moreover, the trend in the levels of phosphorusamongthe different stages was; M at stage I(1023.833mg/kg)>M atstage III>M at stage II(376.250mg/kg). The estimated marginal means (Appendix XII) revealed that phosphorus mean contents decreased with growth of the plant.Besides,the reverse was the case on treatmentsestimatedmarginal means, in which the phosphorus contentsincreased with an increasein mineral fertilizer, F100 having the highest value(627.667mg/kg), followedby F75 and then M accounted for the least value 497.726mg/kg. There was significant difference in the levels of phosphorus among the different stages of growth.However, these differences were statistically insignificant among the treatments at ($p<0.05$). This probably was attributed to the ability of M fertilizer to release its nutrients at slow rate as compared to other mineral fertilizers. Singh and Kapoor, (1999), revealed that, Phosphate solubilizing bacteria (phosphorein) release organic and inorganic acids which reduces soil pH leading to change of phosphorus and other nutrients to available

forms ready for uptake by the plants. This can be manage while increasing the concentration of the mineralfertilizer as demonstrated that 100kg fertilizer contributed higher phosphoruscontent, followed by F75 fertilizer relatively.

5.3.3 Potassium (K) contents

The estimated marginal means, Appendix XII,revealedthat the maximum K content was recorded in stage I (3611.111 mg/kg)followed by stage III, and stage II the lowest (2116.667mg/kg). The differencesin potassium contents among the growing stages were statistically significant at ($p<0.05$). On the other hand, the treatments estimated marginal means recorded M treatment with highest K content(2775.000mg/kg), then F100 and F75 with the least concentration of 2275.000mg/kg (Appendix XII). The differencesin potassium contents were statistically insignificant among the treatments at ($p<0.05$). Manure fertilizer also superseded the other fertilizers in terms of K supplyand making it available to plant during the early and maturity stages, while, F75 had the higher K contents at the flowering period.

5.3.4 Soil pH

The pH in a KCl mediumshowsthe value of analysed samples asslightly acidic.The pH also increasedwith days.However, pHlevel at the control soil was found to be acidic (6.60). This may be attributed to the nature of the soil sampled before the application of fertilizer and afterward. In the same way, the pH value increased with an increase inthe fertilizer level, in which F100 had the higher acid value of (6.50), followed by F75 and then M with(6.50). The differences that exist among the stages of growth and the treatments for soil pH were found statistically insignificant at ($p<0.05$). Thefindings here have qualifies manure to be usedforthe cultivation of *H. sabdariffa* plant, because, phosphate solubilizing bacteria (phosphorein) in manure release organic and inorganic acid which reduce soil pH

leading to change of phosphorus and other nutrients to available forms ready for uptake by plants (Singh and Kapoor, 1999).

The pH of soil in water medium revealed that, M treatment dominates othertreatments across the stages (SI-III),as shown in Figure 4.17to 4.19. However,the pH levels (alkaline pH) of the soil decrease with maturity of the plant.The estimated marginal means for soil at stages of growth revealed that,the alkalinity of the soil decreased with days.Nevertheless, the soil pHfor SII and SIII has lower pH value, which might probably resulted from the excessive rainfall that occurs and leaches the soil nutrients (Ball,1999).Thus, fertilizer treatmentsestimated marginal mean(Appendix XII), shows decrease in pH foran increase in fertilizer concentration;M treatment(7.20)>F75(7.001)>F100(7.037). On the other hand, stage I was statistically found significantfrom other stages of growth,and the differences among the treatments wereinsignificant at ($p<0.05$). Theresults further indicatedmanure fertilizer, asthe best fertilizer for the soil alkalinity and a better fertilizer to be adopted in activating*H. sabdariffa* plant.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The findings in this study showthat calyx was a good supplementsource for mineral elements. The mineral fertilizer 100kg/hectarewas the best fertilizer forthe fat contents sourcing maximally across the growing period(11.242 to 30.710%), while, F75 fertilizer was the best fertilizer needed to obtain maximum carbohydrate and calorific values among the fertilizers across the stages.Poultry manure (M) gave the highest in Cu level the most excellent fertilizer tominimize soil alkalinityvalue (7.513 SI and 7.157 SII),whichwas the best soil pH condition for *Hibiscus sabdariffa* to grow well. However, the Pb was not detected at a vegetative stage and recorded least from M fertilizer at SII, which has shown that stage Iwill be the best harvesting time for *Hibiscus sabdariffa* and stage IIif M was the fertilizer used. Moreover, the copper content obtainedwas highest (18.571mg/kg)with poultry manure treatment. However,anti-nutrient contents were found to be within a safe level, thoughthe oxalate content was on the range of 4.593 to 14.423% and are above the permissible limit.Thus, *Hibiscus sabdariffa* is a nutritious plant and could be used in the fight against malnutrition. Nevertheless, poultry manure cancomplementthe mineral fertilizersas there was no significant difference ($p<0.05$), in the performance. Finally,*Hibiscus sabdariffa*can be used to compensate cereal crops that requiremineralfertilizer to grow.

6.2 Conclusion

The plant was found to be a good source of protein due high calorific value contents (>12%). Similarly, it was found that the level of Zn, calorific value and carbohydrate were highest at the plant develops which indicated the nutritive value of this plant.The anti-nutrients contents such as tannin recorded were below the toxicity limit of 76 - 90g/kg acrossstages of the plant

growth.Thehigh oxalate contents were recorded which could be easily removed by blenching and boiling process, which is part of the treatmentto be made for the edible leaves and calyx before used as a diet. High oxalate contents in any plant indicate that it hasa bio-pesticidal property, in this study the level of oxalate was enhanced in plant when poultry manure was used. Moreover, the high Pb concentration recorded in this study at stages II and III of the plant growth clearly indicates that the metal accumulation could be attributed to the proximity of the farm to main road.

6.3 Recommendations

The following recommendations were drawn from the research findings;

i. More research should be conducted on other parameters at different poultry manure proportionsand mineral fertilizer with *Hibiscus sabdariffa*.

ii. Study should be conducted on mineral fertilizer at 75 kg/ha for the energy nutrients yielding; carbohydrate and calorific value.

iii. More investigationsshould be done on vegetable oil enrichment, dry matter and mineralindex in*Hibiscus sabdariffa*by mineral fertilizer at 100 kg/ha concentration.

iv. Further study should be conducted onbio-pesticidal propertiesof *Hibiscus sabdariffa*grownby manure fertilizer that showed higher oxalate content.

REFFERENCES

Abara, A.E. (2003). Tannin content of Dioscorea bulbufera. *J. Chem. Soc*. Niger. 28, 55-56

Abou El-seoud M.A., M.F Abd Elabour and EA Omar, (1997). Productivity of roselle (Hibiscus sabdariffa L.) plant affected by organic waste compost addition to sandy soil Bull *Nati. Res, Cairo, Egypt*, 22:495-505.

Adanlawo,I.G. and Ajibade V.A. (2006). Nutritive value of the two varieties of Roselle (*Hibiscus sabdariffa)* Calyx soaked with wood. *Parkistan Journal of Nutrition*. 5 (6):555-557

Adeolu A.T and Enesi D.O (2013). Assessment of proximate, mineral, vitamin and

phytochemical compositions of plantain (Musa paradisiaca) bract – an agricultural waste, *International Research Journal of Plant Science* 4(7): 192-197, ttp://www.interesjournals.org/IRJPS , (ISSN: 2141-5447)

Adepoju O.T., Adeniji P.O. (2008). Nutrient composition, antinutritional factors and contribution of native pear (*Dacryoides edulis*) pulp to nutrient intake of consumers. *Nig. J. Nutr.* Sci. 29 (2): 15-23.

Agbo J.T. (2004). Proximate nutrient composition of sickle pod. (*Cassia obtusfolia)* leaves and seeds. *Plants Prod. Res. J*. 8: 13-17.

Ahmed, S.K., El-Ghawas E.O. and Aly, A.F. (1998). Effect of dry yeast and organic manure on Roselle plant. Egypt. *J. of Agric. and Reclamation, Giza, Egypt,* 76(3): 1115-11142.

Ajieroh, V. (2010). A Quantitative Analysis of Determinants of Child and Maternal Malnutrition in Nigeria.

Akindahunsi A.A.and Salawu S.O. (2005). Photochemical screening and nutrient-anti-nutrient composition of selected tropical green vegetables. *Afr. J. Biotech.* 4: 497-501.

Akubugwo, I. E., Obasi, N. A., Chinyere G. C. and Ugbogu A. E. (2007). Nutritional and chemical value of *Amaranthus hybridus* L. leaves from Afikpo, Nigeria.*African Journal of Biotechnology*. 6 (24): pp. 2833-2839

Aletor, V.A. (1995). Compositional studies on edible tropical species ofMushrooms. Food Chemistry. 54:265-26

AllenS.E., Grimshaw H.M., Parkison J.A. and Quarmby C. (1974).Chemical analysis of ecological material. *Black.W. Sci.*P:50-317.

Ali,B.H. Al Wabel,N. and Blunden G. (2005). Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa L.*: a review Phytotherapy Research, 19 (5) ; 369–375
CrossRef| View Record in Scopus | Citing articles (126)

Antia B.S., Akpan E.J., Okon P.A.and Umoren I.U. (2006). Nutritive and Anti-Nutritive Evaluation of sweet potatoes (*Ipomoea batatas*) leaves. *Pak. J. Nutr.* 5(2): 166-168.

AOAC, (2010). Official Methods of Analysis. Association of Official Analytical Chemists, 15th Edn., Washington D.C., USA.

AOAC (1999). Methods of Analysis of Association of official Analytical chemists (16th ed). *Washington, D.C.*1: 600-792.

AOAC (1995). Official methods of analysis (16th edn) Association of Official Analytical Chemists. Arlington, V. A. USA

Appel , L.J. , Moore , T.J. , Oberzanek , E. , Vollmer , W.M. , Svetkey , L.P. , Sacks and F.M. (1997). A clinical trial of the effect of dietary patterns on blood pressure . *N. E. J. Med.*,336: 1117 – 1124 .

Arnell N.W. (2004). Climate change and global water resources: SRES emissions and socio-economic scenarios. Global Environmental Change-Human and Policy Dimensions.14:31-52. PubMed | Google Scholar

Arredondo , M. and Nunez , M.T. (2005) . Iron and copper metabolism. *Mol. Aspects Med.,* 26, 313 – 327

Asibey-Berko E.and Tayie F.A.K.(1999). Proximate analysis of some underutilized Ghanaian vegetables. *Ghana J. sci.* 39: 91-92.

Atta Aly, M.A. (1998). Soaking summer squash seeds in low concentrations of cobalt solution before sowing increased plant growth, femaleness and fruit yield via increasing plant ethylene level. *J. Plant Growth Regul.* 17: 25-32. B

Atta M.1, Idris A, Bukhari Aand Wahidin S.(2013). Intensity of blue LED light: a potential stimulus for biomass and lipid content in fresh water microalgae Chlorella vulgaris. *Bioresour Technol.* 148:373-8. doi: 10.1016/j.biortech.2013.08.162.

Atta,M.B. and ImaizumiK. (2002). Some Characteristics of Crude oil extracted from Roselle (*Hibiscus sabdariffa L.*) seeds cultivated in Egypt,*Journal of Oleo Science*, 51 (7), pp. 457–461
View Record in Scopus | Citing articles (8)

Atta S., Diallo, A.B,Bakasso, Y.,Sarr,B.,Saadou,M.andGlew,R.H.(2010). Micro-element contents in roselle (*Hibiscus sabdariffa* L.) at different growth stages. *African Journal of Food Agriculture, Nutrition and Development.*10(5):2615-2628.

Ayoola P.G. (2011). Determination of proximate composition, vitamins, phytochemical and mineral contents of *Musa paradisiacal* (plantain) root. *P.A Sci. Tech.* 1(2): 16-29.

Aziz, E., Nadia Gad and K. Morssy, (2011).Effect of cobalt on growth and chemical composition of peppermint plant grown in newly reclaimed soils.*Australian Journal of Basic and applied Science,* (In Press).

Aziz E., Gad N.and Badran N. M. (2007). Effect of Cobalt and Nickel on Plant Growth, Yield and Flavonoids Content of *Hibiscus sabdariffa* L. *Aust. J. BasicAppl. Sci.* 1(2): 73-78.

Babalola S. O., Babalola A. O. and Aworh O. C. (2001). Compositional attributes of the calyces of roselle (*Hibiscus sabdariffa*), *J. Food Technol. Afr*. 6(4): 133–134.

Bako, I.G., Mabrouk, M.A. and Abubakar A. (2009). Antioxidant effect of ethanolic seed extract of Hibiscus sabdariffa Linn (Malvaceae) alleviate the toxicity induced by chronic administration of sodium nitrate on some haematological parameters in Wistars rats Advance *Journal of Food Science and Technology*, 1 (1): 39–42View Record in Scopus | Citing articles (10)

Blair N., Faulkner R.D., Till A.R.and Poulton P.R. (2006) Long-term management impacts on soil C, N and physical fertility part I: Broadbalk experiment. Soil Till Res 91: 30–38.

Ball,J.(1999).Understanding-and-correcting-soil-acidity. https://www.noble.org/news/publications/ag-news-and views/1999/january/ /accessed 19/6/2017

Basu, M.P., BhadoriaB.S. and mahapatra,S.C. (2006). Influence of microbial culture in combination with micronutrient in improving the groundnut productivity under alluvial soil of India. *ActaagriculturaeSlovenica*, 87(2): 435- 444.

Briat, J.F. andLobreaux, S. (1997) . Iron transport and storage. *Trends Plant Sci*., 2 , 187 –193 .

Brooke B. (2002) Nutritional Assessment of Children EnrolledIn a Structured Childcare Setting. Pp 1

Chadha, M.A. Oluoch, A.R. Mtukuso, and Daudu A.T.(2000).Vegetable research and development in Malawi, Review and planning workshop proceedings. Pp 1-124

Chewonarin, T. Kinouchi, T. Kataoka, K. Arimochi, H. Kuwahara, T. Vinitketkumnuen U. (1999). Effects of roselle (Hibiscus sabdariffa Linn.), a Thai medicinal plant, on the mutagenicity of various known mutagens in Salmonella typhimurium and on formation of aberrant crypt foci induced by the colon carcinogens azoxymethane and 2-amino-1-methyl-6-phenylimidazo[4,5b]pyridine in F344 rats *Food and Chemical Toxicology*, 37 (6): 591–601
Article| PDF (223 K)| View Record in Scopus | Citing articles (76)

Cisse M., Dornier M., Sakho M., Diop C. M., ReyneS. M. and Sock O. (2009a). La productiondebissap (*Hibiscus sabdariffa*) au Sénégal. *Fruits* 64: 111–124

Cisse M., Dornier M., Sakho M., Ndiaye A., Reynes M. and Sock O. (2009b). Le bissap (*Hibiscus sabdariffa*L.): composition et principales utilisations. *Fruits* 64 (3): 179–193

Cohen, A.J.and Roe, F.J.C. (2000). Review of risk factors for osteoporosis with particularreference to a possible aetiological role of dietary salt.*Food Chem. Toxicol.,* 38: 237 – 253.

Compton, J.E and Boone, R.D. (2002) Soil nitrogen transformation and the role of light fraction organic matter in forest soils. *Soil Biol Biochem* 34: 933–943. PubMed/NCBI

Dickson, R.A., Annan, K. Fleischer, T.C., Amponsah, K.N. and Oteng, J.A (2012). Phytochemical Investigations and Nutritive Potential of Eight Selected Plants from Ghana. *Journal of Pharmacy and nutrition Sciences*, 2(2):2-177.

Dung, N.X. Khien, P.V. Nhuan, D.D. Hoi, T.M. Ban, N.K. and Leclercq P.A. (1999). Composition of the seed oil of Hibiscus abelmoschus L. (Malvaceae) growing in Vietnam *Journal of Essential Oil Research*, 11 (4): 447–452 CrossRef| View Record in Scopus | Citing articles (6)

EcoCrop. (2007). *Hibiscus sabdariffa* var. sabdariffa. In Food and Agriculture Organization of the UN (FAO).

Edmeades DC (2003) The long-term effects of manures and fertilizers on soil productivity and quality: a review. *Nutr Cycl Agroecosyst* 66: 165–180.

El-Adawy T. A. and A. H. Khalil, (1994). Characteristic of roselle seeds as a new source of protein and lipid. *J. Agric.Food Chemist,*42: 1896-1900.

Elamin, K.M., Hassan, H.E., AbdallaA., Tameem, H.O. and Eldar A.A. (2012).Effect of feeding crushed Roselle seed (*Hibiscus sabdariffa L.*) (Karkadeh) on carcass characteristics of Sudan desert sheep Asian *Journal of Animal Science*, 6 (5): 240–248 CrossRef| View Record in Scopus | Citing articles (2)

EL-keltawi,N.E.,A.A. Tawfik.A.M. and Ahmad, (2003).Roselle(*Hibiscus sabdarffa L.*) Production as effected by natural alternative to farmyard manure, II-chemical assessment of sepal quality. *Assuit .J.Agric Sci*.,34:307-321.

Eslaminejad, M. and Zakaria T. (2011). Morphological characteristics and pathogenicity of fungi associated with Roselle (*Hibiscus Sabdariffa*) diseases in Penang Malaysia. *Microbial Pathogenesis*, 51 (5): 325–337 Article| PDF (3010 K)| View Record in Scopus | Citing articles (4)

FAO. (2008). The State of Food Insecurity in the World 2008. Food and Agriculture Organization, Rome. FAO/WHO/UNU. (1985). Protein and Energy Requirements, Food and Agriculture Organization, World Health Organization, United Nations University, Rome.

FAO (2007). The state of Food and Agriculture: Paying farmers for environmental services. Agricultural division (ESA) FAO, htt:www.fao.org/inpho/content/compend/text/ch28.htm. Retrieved 2007 08-25.

FAO (2004). HIBISCUS: Post-Production Management for Improved Market Access. Post-harvest compendium. Rome, Italy. pp 4.

FAO/WHO(2001). Expert Consultation on Allergenicity of Foods Derived from Biotechnology, January 2001 Evaluation of Allergenicity of Genetically Modified Foods Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology 22 – 25 January 2001 Food and Agriculture Organization of the United Nations (FAO) Rome, Italy.

Faruq U.Z, Sani A.and Hassan L.G (2002). Proximate composition of sickle pod. (*Senna obtusfolia*) leaves. *Niger. J.Basic Appl. Sci.* 11: 157- 164.

Gaya, I.B. Mohammad, O.M.A. Suleiman, A.M. Maje, M.I. and Adekunle A.B. (2009). toxicological and lactogenic studies on the seeds of *Hibiscus Sabdariffa* Linn (Malvaceae) extract on serum prolactin levels of albino wistar rats. *The Internet Journal of Endocrinology*, 5 (2).

George E., Rolf S. and John R. (2013). Methods of Soil, Plant, and Water Analysis**:** A manual for the West Asia and North Africa region, ICARDA, Beirut, Lebanon. Pp. 65-66.

Gibbon, D. and Pain A. (1985). Crops of the drier regions of the tropics (1st ed.)English Language Book Society, Longman, England

Groff, J. L., andGropper, S. A. S. (2000). *Advanced nutrition and human metabolism*. Belmont, CA, West/Wadsworth. Pp. 584.

Grubben, G.H. and Denton,O.A.(2004).*Plant Resources of Tropical Africa Vegetables*.PROTA FOUNDATION.TheNetherlands,Technical Centre for Agriculture and Rural Cooperation.Pp :321-327.

Gupta, S., Lakshmi, A.J., Majunath, M.N. and Prakash, J. (2005). Analysis of nutrient and anti-nutrient content of under-utilized green leafyvegetables. *LWT-Food Sci. Tec.* 38: 339-345.

Hambidge, K.M., Casey, C. W., Krebs, N. F. (1987). Zinc: In W. Mertz (ed). Trace elements in human and anim. *I Nutr.5th ed. Academic press Inc., San Diego*, 2, 50-51.

Hassan, L.G.and Umar, K.J. (2006). Nutritional value of Balsam Apple (*Momordica balsamina* L.) leaves. *Pak. J. Nutr.* 5(6): 522-529.

Hiza, H.A.B.and Bente, L. (2007). Nutrient content of the U.S. food supply, 1909–2004: A summary report. Home Economics Research Report Number 57. U.S. Department of Agriculture, Center for Nutrition Policy and Promotion, Washington, DC, USA.

Hughes A.J. Daniel S.E. Kilford L. and Lee A.J.(1992).Accuracy of clinical diagnosis of idiopathic Parkinson's disease. *J. of Neurol. Neurosurg Psychiatry*. 55(3):181-4.

Ibrahim M. M. and Hussein R. M. (2006). Variability, Heritability and Genetic Advance in some Genotypes of Roselle. *World Journal of Agric.Sc.* 2(2): 340-345.

Ibrahim, U., Mukhtar, A. A., Babaji, B. A. and Adepke, D. I. (2014) Effect of poultryManure and weed control methods on growth and yield of Three Groundnut (*Arachis hypogaea* L.) Varieties at Samaru, Zaria, 10(2):18-22

Ifon E.T.and Bassir O. (1979). The nutritive value of some Nigerian leafy green Vegetables-Part 1: Vitamin and mineral contents. *Food Chem.* 4: 263-267.

Ignarro, L.J., Balestrieri, M.L.and Napoli, C. (2007). Nutrition, physical activity, and cardiovasculardisease: An update. *Cardiovasc. Res.*, 73: 326 – 340.

Institute of Medicine Food and Nutrition Board Dietary (1999). Reference Intake:Calcium, Phosphorus, Magnesium, Vitamin D and Flouride. National Academy Press, Washington, DC 1999.

Inuwa H.M., Aina, V.O., Aimola, B.G.I. and Amao, T. (2011). Comparative Determination of Anti-nutrient Factors in GroundnutOil, Palm Oil.*Advanced Journal of Food Science and technology,* 3(4): 275-279.

Ishida A., Tanabe H., Nakano T., Koike N., Uemura A. and Yamashita n. (2000). Light-use properties in two sun-adapted shrubs with contrasting canopy structures. *Tree Physiology*, 21, 497–504

Ismail, A. Ikram, E.H.K. and Nazri H.S.M. (2008). Roselle (Hibiscus sabdariffa L.) seeds nutritional composition protein quality and health benefits Food, 2 (1): 1–16
View Record in Scopus | Citing articles (13)

Isong E.U., Adewusi S.A.R., Nkanga E.U., umoh E.E.and Offiong E.E. (1999). Nutritional and phytogeriatological studies of three varieties of *Gnetum africanum*(afang). *Food Chem.* 64: 489-4.

Jodral-Segado, A.M., Navarro-Alarcon, M. , Lopez-G de la Serrana, H.and Lopez Martinez, M.C. (2003) . Magnesium and calcium contents in foods from SE Spain: Infl uencing factors and estimation of daily dietary intakes. *Sci. Total Environ.* , 312:47 – 58.

Johnston L. M., Moe C. L., Moll D. and Jaykus L. (2006).The epidemiology of produce-associated outbreaks of foodborne disease. In: Microbial Hazard Identification in Fresh Fruits and Vegetables (edited by J. James). New Jersey: John Wiley and Sons. Pp. 38-52.

Kelsay J.L., Behall K.M. and Prather, E.S.(1979).Effect of fiber from fruits and vegetables on metabolic response of human subjects II. Calcium, magnesium, iron and silicon balances. *American journal of clinic nutrition,* 32:1876-1880

Kennedy, E., Bowman, S. and Powell, R.(2001). Assessment of the effect of fat-

modified foods on diet quality in adults, 19–50 years, using data from the Continuing Survey of Food Intake by Individuals. J. *Am. Diet. Assoc.*, 10, 455 – 460 .

Korcak, R.F. (1987). Iron deficiency chlorosis. *Hort. Rev*., 9, 133 – 186 .

Laila, M. H. and Nadia G. (2002).Effect of cobalt fertilization on the yield, quality and the essential oil composition of parsley leaves. *Arab Univ. J.Agric. Sci. Ain Shams,* cairo, 10(3): 803-829.

Ladan M.J., Bilbils L.S.and Lawal M. (1996). Nutrient composition of some green leafy vegetable consumed in Sokoto. *Niger. J.Basic Appl. Sci.* 5: 39-44.

Leung, A.Y. and Foster S. (1996). Encyclopedia of common natural ingredients used in food, drugs, and cosmetics (2nd ed.)John Wiley and Sons, New York

Lockeett C.T., Calvert C.C.and Grivetti L.E. (2000). Energy and micronutrient Composition of dietary and Medicinal wild plants Consumed during drought: Study of Rural Fulani, Northeastern Nigeria. *Int. J. Food Sci. Nutr.* 51: 195-208.

Lokeshappa B., Kandarp S., Vivek Tripathi and Anil K. D (2012).Assessment of Toxic Metals in Agricultural Produce,*Food and Public Health*,2(1): 24-29 doi:10.5923/j.fph.20120201.05

Lorenzo D., Atti-Serafini L., Santos A., Frizzo C. D., Paroul N., Paz D., Dellacassa E. and Moyna P. (2000). Achyrocline satureioid essential oils from southern Brazil and Uruguay.*Planta Medica* 66: 476–477.

Luchuo E. B., Paschal K. A., Ngia G., N. Peter K., Yelena S., Nsah B.and Ajime T. T. (2013). Malnutrition in Sub–Saharan Africa: burden, causes and prospects. *The Pan African Medical Journal.*2013(15):120.

MacDonald , H.M. (2007) . Influence of organic salts of potassium on bone health:Possible mechanisms of action for the role of fruit and vegetables .*Int. Congr. Ser.*,1297, 268 – 281 .

Mahran, G.H. El-Hossary, G.A. and El-Labban H.M. (1979). The effect of main nutrient elements on the growth and yield of roselle plant –*Hibiscus sabdariffa L*. *Acta Pharmaceutica Hungarica*, 49 (1): 2–8
View Record in Scopus | Citing articles (1)

Murdock, G.P. (1959). Murdock Africa, its peoples and their culture history McGraw-Hill, New York (1959)

Matthews K. R. (2006). Microorganisms associated with fruits and vegetables. In: Microbiology of Fresh Produce (edited by K. R. Matthews). Washington DC: ASM Press. Pp. 1-21.

Mathew C. S. (1985): Microbiological quality of food product. *Journal of food Protection 654* (4): 304 – 309.

McCaleb R.S. (2000). Market survey: Hibiscus sabdariffa. Agribusiness in sustainable natural African plant products Herb Research Fondation.
http://www.herbs.org/africa/hibiscus.html. Accessed in October 2012

McCarron, D.A.and Reusser, M.E. (2001). Are low intakes of calcium and potassium important causes of cardiovascular disease?. *Am. J. Hyp*. 14: S206 – S212.

McClintock N. C. and El Tahir I. M. (2004).*Hibiscus sabdariffa* L. In: Grubben, G. J. H. and Denton O. A. (Editors). PROTA 2: Vegetables. [CD-Rom].PROTA, Wageningen, Netherlands.

McCluggage, D. (1991). Heavy metal poisoning, NCS, magazine, published by the bird hospital, Co. USA. In: Seasonal variations of heavy metal concentrations in abbatoir dumping site soil in Nigeria. *Journal of Applied Science and Environmental Management*, 13 (4), 9-13

Mengel, K.,and Kirkby, E.A. (1982). Principles of Plant Nutrition. International Potash Institute, Worblaufen-Bern, Switzerland

Mohamed, B.B. Sulaiman, A.A. and Dahab A.A. (2012). Roselle (*Hibiscus sabdariffa L.*) in Sudan, cultivation and their uses Bulletin of Environment, Pharmacology and Life Sciences, 1 (6):48–54 View Record in Scopus | Citing articles (7)

Morton J. (1987). Roselle. In: Morton J, editor. Fruits of warm climates. Miami: Creative Resources systems Inc. p 281-286.

Mounts T.L. (2000).The Chemistry of Components, 2nd Edn. Royal Society of Chemistry.

Mukhtar M.A. (2007).The effect of feeding rosella (Hibiscus sabdariffa) seed on broiler chick's performance. *Research Journal of Animal and Veterinary Sciences*, 2: 21–23 View Record in Scopus | Citing articles (6)

Munro and Basir,O. (1969). Oxalate in Nigeria vegetables. *W. Afr. J. Biol. Appl. Chem*., 12: 14-18.Murdock, G.P. (1959). Africa, its peoples and their culture history McGraw-Hill, New York

Nabila, Y.N. and M.S. Aly, (2002). Variations in productivity of (Hibiscus sabdariffa L.) in response to some agricultural supplementation. *Annals of Agric. Sci*. (Cairo), Faculty of Agric. Ain Shams Univ. Cairo, Egypt., 47(3): 875-892.

Nadia G., (2010). Improving quantity and quality of canola oil yield through cobalt nutrition.*Agric. Biol. J. N. Am.*, 1(5): 1090-1097.

Nadia G. and Hala K.(2008). Response of sweet potato (Ipomoea batatas L.) plants to different levels of cobalt. Australian J. Basic and Applied Sci 2(4): 949- 955.

Neuwinger, H. (2000). African traditional medicine Medpharm Scientific Publication, Stuttgart(2000)

Nwachukwu, N and Obi, C.E. (2007). Comparative studies on the Effect of open Air and oven drying on the Anti-nutrient content of some leafy vegetables of Eastern. Nigeria.*Bio-Research* 5(1): 216-220.

Nyarko G., Bayor H., Craigon J. andSuleimana I.. (2006). The effect of container types, seed dressings and desiccants on the viability and vigour of roselle (*Hibiscus sabdariffa* L. var. *sabdariffa*) seeds. Pak. *J. Biol. Sci.* 9(4): 593–597.

Nzikou, J.M. Bouanga-Kalou, G. Matos, L. Ganongo-Po, F.B. Mboungou-Mboussi, P.S. and Moutoula, F.E. (2011). Characteristics and nutritional evaluation of seed oil from Roselle (*Hibiscus sabdariffa L.*) in Congo-

Olarinwaju R.B. Funke I. O., Segun B. F. and Foluke E. S. (2011). Prevalence and Determinantsof Malnutrition among Under-five Children of Farming Households in Kwara State, Nigeria, 3(3)173-181 doi:10.5539/jas.v3n3p173

Parr, J.F. and S.B. Hornick,(1990). Recent development in alternative agriculture in the United State. In pro.of In thl conf. on kyussei nature forming. October 17-29, 1989, Khankean Univ Thailand.

Pearson D (1976). Chemical analysis of foods. (7th ed) Churchchill, Livingstone, London, Pp. 218-336.

Plotto, A. (2004). Hibiscus: post-production management for improved market access. In: Food and Agriculture Organization of the UN (FAO).

Rao C.V., Newmark H.L. and Reddy B.S.(1998). Chemo-preventive effect of squalene on colon cancer.*Carcinogenesis*. 19(2):287-90.

Rao P.U.(1996). Nutrient composition and biological evaluation of mesta (Hibiscus sabdariffa) seeds Plant. *Foods for Human Nutrition*, 49 (1): 27–34View Record in Scopus | Citing articles (47)

Robinson, D.S., (1990). Food Biochemistry and Nutritional Value. Longman Scientific and Technical Publisher, New York, USA.

Romheld, V.and Marschner, H. (1991). Function of micronutrients in plants. In: Micronutrients in agriculture , J.J. Mortvedt , F.R. Cox , L.M. Shuman , R.M. Welch (eds),2nd edn. Soil Science Society of America, Madison, WI, USA, Pp. 297 – 328 .

Roth A.R.and Townsend C.E. (2003). Nutrition and diet therapy 8th edn. Delmar Learning, Thomson Learning Inc. Canada, Pp.132.

Ruan, Q.Y., Zhenga, X.Q., Chena, B.L., Xiao, Y. P., David, X.X., Leung, W.M. and Liu, E.E.(2013). Determination of total oxalate contents of a great variety of foods commonly available in Southern China using an oxalate oxidase prepared from wheat bran. *Journal of Food Composition and Analysis*,32:6–11.

Tuazon, N.T, and Savage G. (2013). Investigation of Oxalate Levels in Sorrel Plant Parts and Sorrel-Based Products,*Food and Nutrition Sciences*, 4, 838-843 http://dx.doi.org/10.4236/fns.2013.48109

Runner, R.S., Kaual,M.K. and Jain,J.C. (2003).Role of trace elements in tomato plants. *Pl. Physiol*., 66: 420- 423.

Salmah, Y., Peng-Kong H., Mohd G. and Yaakot bin C. (2003) Optimization of hot water extraction of roselle juice using response surface methodology: a comparative study with other extraction methods. *J. Sci of Food and Agric*. 83: 1273 – 1278.

Salunkhe, D.K., Bolin, H.R.and Reddy, N.R. (1991). Storage, processing and nutritional quality of fruits and vegetables. Volume I. Fresh Fruits and Vegetables .CRC Press, Boston, MA, USA .

Seguchi, M.and Hayashi, M. (1998). Iron-enriched bread with karkade (*Hibiscus sabdariffa*) and wheat flour. *Cereal Chem* 75 (5):686 – 689.

Sena, L.P. VanderJagt, D.J., Rivera C., Tsin A.T.C., Muhammadu, I., Mahammadu, O., Milson, M.,Pastosyn, A.and Glew, R.H. (1998). Analysis of Nutritional Components of eight famine foods of the Republic of Niger.*Plant Foods Human.Nutrition.* 52: 17-30.

Sigman-Grant , M. , Warland , R. and Hsieh , G. (2003) . Selected lower-fat foods positively impact nutrient quality in diets of free-living Americans, *J. Am. Diet. Assoc*., 103, 570 – 576 .

Smith, L.C. and Haddad, L. (1999). Explaining Child Malnutrition in Developing Countries: Across-Country Analysis. IFPRI FCND Discussion Paper No. 60, IFPRI, Washington, D.C. USA.

Singh, S. and Kapoor K.K. (1999). Inoculation with phosphate-solubilizing microorganisms and visicular- arbuscular mycorrhizal fungus improves dry matter yield and nutrient uptake by wheat grown in sandy soil. *Biol. Ferti. Soils,* 28: 139-144.

Soetan, K.O., Olaiya, C.O. and Oyewole, O.E. (2010). The Importance of Mineral Elements for Humans, Domestic Animals and Plants: A Review. *African Journal of Food Science,* 4, 200-222.

Sowicki, B.(2003). The influence of cobalt fertilization on quantity and quality of hay from dried meadow using various NPK doses. Annals Univ. Mariae Curie Skloodoska Section-E. Agric., 54: 97-104.

Stoltzfus, R.J.and Dreayfus, M.L. (2012). Guidelines for the use of Iron supplement to prevent and treat Iron deficiency anaemia, International Nutritional Anaemia ConsultancyGroup (INNCG).http://www.who.int/nutrition/publications/micronutrients/guidelines-for-Iron-suplimentation. Accessed 19/06/2015.

Swietlik, D. (1999). Zinc nutrition in horticultural crops. Hort. Rev., 23 , 109 – 178 .

Taura D.W.and Habibu A.U.(2009). Bacterial contamination of Lactucasativa, Spinaciaolerencea and Brassica olerencea in Kano Metropolis. *Int. J. Biomedand hlth Sc.* **5**(1): 6.

Tressler, D.K., Van Arsdel, W.B.and Copley M.J. (1980). The freezing preservation of foods, 4th edn, Vol 23, AVI Publishing Co. Westport, Conn

Udedi, S. S. (2003). From guinea worm scourge to metal toxicity in Ebonyi State.Chemistry in Nigeria as the New Millennium Unfolds, 2: 13-14. In: Heavy metal contents in soil medicinal plants in high traffic urban area. *Pakistan Journal of Nutrition*, 10 (7), 618 – 624.

UNDP, (2017).Roselle demand globally. oalsfile:///D:/download/roselledemandglobally.html. Accessed 1/4/2017.

Uriu-Adams, J.Y. and Keen, C.L. (2005). Copper, oxidative stress, and human health . *Mol. Aspects Med.*, 26; 268 – 298.

USDA, (2007). A. USDA National Genetic Resources Program Taxon: Hibiscus sabdariffa L.Germplasm Resources Information Network – (GRIN) [Online Database], National Germplasm Resources Laboratory, Beltsville, Maryland (2007) http://www.ars-grin.gov/cgibin/npgs/html/taxon.pl?19078

Van de Poel, E., Hosseinpoor, A., Jehu-Appiah, C. and Speybroeck, N. (2007). Malnutrition and Socioeconomic Gap in Malnutrition in Ghana. Mimeo.

Wahid, A., Farooq M. and Kadambot H. M. S. (2012).Micronutrient application through seed treatments - a review, *Journal of Soil Science and Plant Nutrition*, 12 (1), 125-142
http://dx.doi.org/10.4067/S0718-95162012000100011

Wallstrom, P., Wirfalt, E., Janzon, L., Mattisson, I., Elmstahl, S., Johansson, U., and Berglund, G.(2000). Fruit and vegetable consumption in relation to risk factors for cancer: a report from the Malmo Diet and Cancer Study. *Public Health Nutr*.3: 263.

Wang, M.L. Morris, B. Tonnis, B. Davis, J. and Pederson G.A. (2012). Assessment of oil content and fatty acid composition variability in two economically important Hibiscus species,*Journal of Agricultural and Food Chemistry*, 60 (26): 6620–6626CrossRef| View Record in Scopus | Citing articles (7)

Wang H., Cao G.and Prior R.L. (1997). Oxygen radical absorbing capacity of anthocyanins.*J. Agric. Food Chem*. 45: 304-309.

Watson J. W. and Eyzaguire P. B. (eds.) (2002) Home gardens and in situ conservation of plant genetic resources in farming systems.Proc.2nd Int. Home Gardens Workshop.17-19 July 2001, Witzenhausen, Federal Republic of Germany.3-9. *Wester P. J.* (Philipine Agr. Rev.[English Ed.], 7(1914), No. 6 Pp.266-269, pls 3).

Werner, W. and Josef, B.(2002). Solar drying. Establishement of production, sales and consulting infrastructure for solar thermal plants in Zimbabwe. Institute of sustainable technologies, Austria., 1-35 .

Whitney, E. and Rolfes, S.(1999). Understanding nutrition. Belmont,Ca. USA, West/Wadsworth. Eighth ed. (ed. W. Rolfes). Pp 342.

Wilson, F.D. and Menzel M.Y. (1964). Kenaf (*Hibiscus cannabinus*), roselle (*Hibiscus sabdariffa*)*Economic Botany*, 18(1): 80–91CrossRef| View Record in Scopus | Citing articles (39)

WHO (1993).World Health Organization Guidelines for Technologies for Water Supply SystemsinSmallCommunities.Genevarecommendations,Vol.1

World Health Organization (2003). Post-harvest and pressing technology of staple food. Technical compendium of WHO Agricultural Science Bulletin 88:171-172.

World Health Organization (WHO); Geneva, (1996) and Geneva, 2, 170-187.

Yagoub Ael, G. Mohamed, B.E. Ahmed, A.H. and El Tinay A.H. (2004). Study on furundu, a traditional Sudanese fermented roselle (*Hibiscus sabdariffa L.*) seed: Effect on in vitro protein digestibility, chemical composition, and functional properties of the total proteins *Journal of Agricultural and Food Chemistry*, 52 (20): 6143–6150

Yajnik C.S. and Desmukh W. (2008). Maternal nutrition, intrauterine programming and consequential risks in the offspring. *Rev. Endocr Metab Disord*., 9 (3):203-11.

Zerwekh, J.E., Odvina, C.V., Wuermser, L.-A. and Pak, C.Y.C. (2007) . Reduction of renal stone risk by potassium-magnesium citrate during 5 weeks of bed rest. *J. Urol.,* 177: 2179 – 2184.

APPENDICES

Appendix I: Mean percentage and standard deviation for proximate and anti-nutrients constituents of *Hibiscus sabdariffa* at its growing stages and Calyx.

Treatments/ Parameters	%Moisture content	%Ash content	%Crude protein	%Crude fat	%Crude fiber	%Carbohydrate	Calorific value(kcal/100)	%Oxalate	%Tannin
SI									
M	6.812 ±0.253	12.411 ±0.600	20.910 ±2.132	15.455 ±2.428	12.047 ±0.684	32.366 ±5.555	267.400 ±6.948	9.978 ±2.359	2.685 ±0.068
F75	6.989 ±1.053	12.742 ±0.999	15.322 ±5.568	14.402 ±4.712	11.47 ±9.462	42.223 ±12.184	276.445 ±16.411	8.455 ±3.364	2.756 ±0.114
F100	6.504 ±0.354	13.130 ±2.564	18.426 ±0.704	30.710 ±0.054	11.173 ±0.0542	41.960 ±7.657	288.276 ±35.572	8.190 ±0.213	2.736 ±0.454
SII									
M	4.734 ±3.607	11.351 ±0.656	13.117 ±1.815	9.431± 0.679	13.233 ±2.422	51.917 ±4.211	328.112 ±9.668	14.423 ±0.387	2.442 ±0.411
F75	4.734 ±3.854	9.653 ±2.313	11.608 ±1.512	9.259± 1.364	12.543 ±0.761	55.431 ±5.286	331.197 ±25.364	7.628 ±0.443	2.857 ±0.371
F100	6.990 ±0.920	10.750 ±0.131	9.761 ±4.443	11.242 ±5.369	10.867 ±5.452	53.389 ±8.964	305.367 ±22.426	11.904 ±2.168	2.610 ±0.151
SIII									
M	9.319 ±2.545	8.905 ±1.194	5.7003 ±1.927	11.510 ±1.143	10.160 ±0.308	54.405 ±3.696	293.174 ±13.326	5.931 ±0.618	2.408 ±0.672
F75	5.751±0.282	8.971±9.056	3.667±1.842	13.337 ±0.921	10.413± 2.134	57.860±5.927	302.668±30.606	10.318 ±0.978	3.344±0.910
F100	2.469 ±2.203	11.171 ±3.745	8.708 ±3.068	15.652 ±3.922	11.373 ±3.106	50.627 ±11.232	297.180 ±34.989	6.028 ±0.845	3.704 ±0.759
CALYX									
M	7.433 ±0.131	6.965 ±0.296	3.069 ±0.320	11.813 ±0.644	10.280 ±0.326	60.440 ±1.177	309.302 ±3.796	8.867 ±1.422	5.230 ±3.012
F75	7.524 ±0.449	6.581 ±0.054	2.986 ±0.975	10.028 ±2.337	11.157 ±0.924	61.725 ±3.375	321.023 ±4.851	8.481 ±1.771	4.443 ±0.377
F100	8.032 ±0.266	7.0753 ±1.749	3.335 ±1.312	14.094 ±2.272	10.967 ±0.837	56.497 ±1.591	301.622 ±6.859	9.428 ±1.714	4.190 ±0.346

Appendix II: Mean concentration (mg/kg) and standard deviation for minerals components of *Hibiscus sabdariffa* at its growing stages and Calyx.

Treatments / Elements	Na	Mg	K	Ca	Mn	Fe	Zn	Pb	Cu
SI									
M	448.903	7881.915	10563.292	33192.429	714.810	750.311	30.689	BDL	18.571
	±1.55	±14.64	±1.40	±103.13	±0.10	±2.30	±0.02		±0.04
F75	114.785	6158.574	10399.679	13597.588	586.839	922.508	30.150±	BDL	10.428
	±0.32	±2.56	±2.31	±18.07	±0.80	±2.49	0.04		±0.01
F100	339.001	4316.644	6173.347	23600.721	781.717	1377.294	21.833	BDL	13.222
	±1.84	±4.85	±0.56	±25.90	±1.23	±6.54	±0.10		±0.04
SII									
M	267.533	5206.236	11113.030	15298.459	727.214	878.305	53.942	0.948	6.983
	±0.29	±3.42	±2.39	±24.99	±0.31	±0.80	±0.15	±0.04	±0.00
F75	53.628	6108.076	7625.272	29249.161	857.639	531.562	31.815	13.599	8.317
	±0.10	±9.90	±0.82	±45.77	±1.05	±0.48	±0.12	±0.01	±0.01
F100	81.136	4475.578	8860.880	30995.241	928.713	569.662	21.376	11.357	8.994
	±0.12	±13.59	±2.40	±34.88	±0.55	±0.44	±0.05	±0.02	±0.03
SIII									
M	95.047	5622.268	10907.235	17765.747	635.648	787.385	33.292	20.352	8.628
	±1.38	±4.50	±2.23	±36.31	±0.29	±2.33	±0.04	±0.04	±0.02
F75	155.342	7154.948	7435.793	26719.547	825.470	319.146	30.686	24.249	10.205
	±0.91	±1.13	±0.27	±22.83	±0.99	±0.22	±0.06	±0.01	±0.01
F100	172.662	2771.863	8595.465	28044.460	686.567	627.792	25.995	26.624	5.427
	±0.72	±7.52	±0.47	±68.32	±0.48	±1.15	±0.13	±0.06	±0.01
CALYX									
M	118.356	3801.449	14964.375	18457.987	518.251	105.955	43.740	23.607	4.105
	±0.25	±1.72	±0.36	±22.74	±0.11	±0.05	±0.02	±0.04	±0.01
F75	102.342	4569.806	13718.690	19146.253	463.280	122.934	48.143	22.803	4.397
	±0.27	±5.15	±0.42	±10.20	±0.69	±0.10	±0.07	±0.01	±0.00
F100	142.433	4927.246	17492.187	22252.735	521.139	205.662	59.995	24.201	5.738
	±0.10	±3.08	±0.82	±4.30	±0.64	±0.52	±0.09	±0.003	±0.01
WHO	2500.00	350.00	3500.00	1000.00	600.00	17.00	20.00	0.300	10.00

Appendix III: Mean concentration (mg/Kg) of macro nutrients, percentage moisture, pH levels and standard deviation obtained at three growing stages of *Hibiscus sabdariffa.*

Treatments/ Parameters	N(mg/kg)	P(mg/kg)	K(mg/kg)	Soil PHK	Soil PHW	%Moisture Content
SI						
M	536.667 ±40.415	1023.833 ±417.392	4533.333 ±3082.748	6.640 ±0.0000	7.513 ±0.1550	0.992 ±0.771
F75	350 ±185.203	1153.583 ±625.866	3016.667 ±189.297	6.647 ±0.3086	7.283 ±0.1877	2.491 ±2.114
F100	303.333 ±80.829	1080.667 ±958.329	3283.333 ±778.353	6.527 ±0.2157	7.193 ±0.0322	3.035 ±3.552
SII						
M	210 ±70.000	376.250 ±96.250	2183.333 ±175.594	6.360 ±0.288	7.157 ±0.255	1.487 ±0.516
F75	256.667 ±106.927	646.083 ±546.663	2566.667 ±600.694	6.303 ±0.3653	6.653 ±0.3009	1.091 ±0.829
F100	256.667 ±106.927	522.083 ±227.556	1600 ±229.129	6.523 ±0.1801	7.047 ±0.2570	1.456 ±0.730
SIII						
M	350.185 ±208	393.833 ±105.268	2583.333 ±621.155	6.277 ±0.0252	6.933 ±0.0252	1.677 ±0.262
F75	256.667 ±40.415	414.250 ±169.069	1983.333 ±160.728	6.313 ±0.4042	6.977 ±0.1901	1.763 ±0.057
F100	256.667 ±40.415	710.917 ±641.156	2416.667 ±490.748	6.547 ±0.5235	6.817 ±0.0851	1.763 ±0.057
CONTROL	210±0	197±0	1800±0	6.580±0	7.090±0	0.308±0

Appendix IV: ANOVA multivariate tests for proximate and anti-nutrients compositions of *Hibiscus sabdariffa* cultivatedatdifferent growth conditions and stages

Effect		Value	F	Hypothesis df	Error df	Sig.
Treatments	Pillai's Trace	0.628	1.171	18.000	46.000	0.323
	Wilks' Lambda	0.468	1.130[b]	18.000	44.000	0.358
	Hotelling's Trace	0.933	1.088	18.000	42.000	0.396
	Roy's Largest Root	0.576	1.473[c]	9.000	23.000	0.216
Sample	Pillai's Trace	1.783	3.907	27.000	72.000	0.000
	Wilks' Lambda	0.038	4.955	27.000	64.894	0.000
	Hotelling's Trace	7.963	6.095	27.000	62.000	0.000
	Roy's Largest Root	5.869	15.652[c]	9.000	24.000	0.000

The means difference was at P≤ 0.05

Appendix V: Tests between-subjects effects of proximate and anti-nutrients contents of *Hibiscus sabdariffa* cultivatedat different growth conditions and stages

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatments	MC	7.600	2	3.800	0.724	0.493
	ASH	6.575	2	3.288	0.423	0.659
	CFAT	222.709	2	111.354	1.426	0.256
	CP	63.835	2	31.917	3.157	0.057
	CF	0.173	2	0.086	1.116	0.341
	Carbohy	129.967	2	64.984	1.733	0.194
	Cal. V	474.412	2	237.206	0.455	0.638
	Oxalate	8.102	2	4.051	0.697	0.506
	Tannin	2.640	2	1.320	1.569	0.225
Sample	MC	25.786	3	8.595	1.638	0.201
	ASH	161.161	3	53.720	6.919	0.001
	CFAT	606.872	3	202.291	2.590	0.071
	CP	1039.094	3	346.365	34.264	0.000
	CF	0.603	3	.201	2.598	0.071
	Carbohy	1878.930	3	626.310	16.706	0.000
	Cal.V	5837.978	3	1945.993	3.736	0.022
	Oxalate	70.205	3	23.402	4.027	0.016
	Tannin	11.160	3	3.720	4.420	0.011

The means difference was at P≤ 0.05

Appendix VI: Estimated marginal means of proximate compositions of *Hibiscus sabdariffa*at different growth conditions and stages

Dependent Variable	Samples	Mean	Dependent Variable	Treatments	Mean
Moisture contents	1st sample	6.768±0.763	Moisture contents	M	7.074±0.661
	2nd sample	5.486±0.763		F75	6.250±0.661
	3rd sample	5.847±0.763		F100	5.999±0.661
	Calyx	7.663±0.763	Ash contents	M	9.950±0.804
Ash contents	1st sample	12.761±0.929		F75	9.487±0.804
	2nd sample	10.640±0.929		F100	10.532±0.804
	3rd sample	9.683±0.929	Crude fat	M	12.052±2.551
	Calyx	6.874±0.929		F75	11.754±2.551
Crude fat	1st sample	20.189±2.946		F100	17.173±2.551
	2nd sample	8.975±2.946	Crude protein	M	10.699±0.918
	3rd sample	13.498±2.946		F75	7.609±0.918
	Calyx	11.978±2.946		F100	10.057±0.918
Crude protein	1st sample	17.170±1.060	Crude fiber	M	11.430±0.350
	2nd sample	11.495±1.060		F75	11.396±0.350
	3rd sample	6.025±1.060		F100	11.095±0.350
	Calyx	3.130±1.060	Carbohydrate	M	49.782±1.900
Crude fiber	1st sample	11.563±0.404		F75	54.310±1.900
	2nd sample	12.214±0.404		F100	50.618±1.900
	3rd sample	10.649±0.404	Calorific value	M	299.497±5.866
	Calyx	10.801±0.404		F75	307.833±5.866
Carbohydrate	1st sample	38.850±2.194		F100	298.112±5.866
	2nd sample	53.579±2.194	Oxalate	M	9.800±0.696
	3rd sample	54.297±2.194		F75	8.720±0.696
	Calyx	59.554±2.194		F100	8.888±0.696
Calorific value	1st sample	277.373±6.774	Tannin	M	2.757±0.265
	2nd sample	321.559±6.774		F75	3.350±0.265
	3rd sample	297.674±6.774		F100	3.310±0.265
	Calyx	310.649±6.774			
Oxalate	1st sample	8.874±0.804			
	2nd sample	11.318±0.804			
	3rd sample	7.426±0.804			
	Calyx	8.925±0.804			
Tannin	1st sample	2.726±0.306			
	2nd sample	2.636±0.306			
	3rd sample	3.152±0.306			
	Calyx	4.042±0.306			

The means difference was at $P \leq 0.05$

Appendix VII: ANOVA multivariate tests for minerals components of *Hibiscus sabdariffa* cultivatedatdifferent growth conditionsand stages

Effect		Value	F	Hypothesis df	Error df	Sig.
TREATMENTS	Pillai's Trace	1.046	2.093	22.000	42.000	0.020
	Wilks' Lambda	.224	2.019	22.000	40.000	0.026
	Hotelling's Trace	2.250	1.943	22.000	38.000	0.035
	Roy's Largest Root	1.373	2.621	11.000	21.000	0.028
SAMPLE	Pillai's Trace	2.113	4.765	33.000	66.000	0.000
	Wilks' Lambda	.004	9.568	33.000	59.628	0.000
	Hotelling's Trace	41.913	23.709	33.000	56.000	0.000
	Roy'sLargest Root	39.050	78.100	11.000	22.000	0.000

The means difference was at P≤ 0.05

Appendix VIII: Tests between-subjects effects for minerals components of *Hibiscus sabdariffa*cultivatedatdifferent growth conditions and stages

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
TREATMENTS	Na	95956.516	2	47978.258	3.248	0.053
	K	28774767.313	2	14387383.657	1.599	0.219
	Mg	23671747.234	2	11835873.617	5.164	0.012
	Ca	171242184.591	2	85621092.296	1.136	0.335
	Mn	39216.231	2	19608.115	1.255	0.300
	Fe	310085.631	2	155042.815	1.118	0.340
	Zn	405.967	2	202.984	1.024	0.371
	Pb	587.147	2	293.573	5.797	0.007
	Cu	12.123	2	6.062	0.487	0.619
	Oxalate	8.102	2	4.051	.697	0.506
	Tannin	2.640	2	1.320	1.569	0.225
SAMPLE	Na	197960.353	3	65986.784	4.468	0.010
	K	269573549.452	3	89857849.817	9.986	0.000
	Mg	12848944.101	3	4282981.367	1.869	0.156
	Ca	139451267.157	3	46483755.719	0.617	0.610
	Mn	524561.396	3	174853.799	11.194	0.000
	Fe	3463775.120	3	1154591.707	8.329	0.000
	Zn	2892.585	3	964.195	4.864	0.007
	Pb	18349.281	3	6116.427	120.769	0.000
	Cu	407.040	3	135.680	10.898	0.000
	Oxalate	70.205	3	23.402	4.027	0.016
	Tannin	11.160	3	3.720	4.420	0.011

The means difference was at P≤ 0.05

Appendix IX: Estimated marginal means of mineral compositions of *Hibiscus sabdariffa* cultivatedatdifferent growthconditionsand stages

Dependent Variable	Stages	Mean	Dependent Variable	Conditions	Mean
Na	SI	300.896±40.511	Na	M	232.460±35.083
	SII	134.099±40.511		F75	106.524±35.083
	SIII	135.244±40.511		F100	179.478±35.083
	CALYX	121.044±40.511	K	M	11886.983±865.962
K	SI	9045.439±999.927		F75	9794.858±865.962
	SII	9199.727±999.927		F100	10280.469±865.962
	SIII	8979.498±999.927	Mg	M	5627.967±437.042
	CALYX	15391.751±999.927		F75	5997.851±437.042
Mg	SI	6119.044±504.653		F100	4122.833±437.042
	SII	5263.297±504.653	Ca	M	21178.656±2506.560
	SIII	5183.026±504.653		F75	22178.137±2506.560
	CALYX	4432.834±504.653		F100	26223.289±2506.560
Ca	SI	23463.580±2894.326	Mn	M	648.981±36.080
	SII	25180.954±2894.326		F75	683.307±36.080
	SIII	24176.585±2894.326		F100	729.534±36.080
	CALYX	19952.325±2894.326	Fe	M	630.489±107.478
Mn	SI	694.455±41.661		F75	474.038±107.478
	SII	837.855±41.661		F100	695.102±107.478
	SIII	715.895±41.661	Zn	M	40.416±2.054
	CALYX	500.890±41.661		F75	35.199±2.054
Fe	SI	1016.704±124.105		F100	32.299±2.054
	SII	659.843±124.105	Pb	M	1.778±2.054
	SIII	578.107±124.105		F75	4.938±2.054
	CALYX	144.851±124.105		F100	11.476±2.054
Zn	SI	27.558±4.693	Cu	M	9.572±1.019
	SII	35.711±4.693		F75	8.337±1.019
	SIII	29.990±4.693		F100	8.345±1.019
	CALYX	50.626±4.693			
Pb	SI	-31.551±2.372			
	SII	8.529±2.372			
	SIII	23.742±2.372			
	CALYX	23.537±2.372			
Cu	SI	14.073±1.176			
	SII	8.098±1.176			
	SIII	8.087±1.176			
	CALYX	4.747±1.176			

The means difference was at $P \leq 0.05$

Appendix X: ANOVA multivariate tests of physico-chemical parameters of the soilusedto grow *Hibiscus sabdariffa*atdifferentconditionsandstages

Effect		Value	F	Hypothesis df	Error df	Sig.
TREATMENTS	Pillai's Trace	0.293	0.927	10.000	54.000	0.516
	Wilks' Lambda	0.714	0.952[b]	10.000	52.000	0.495
	Hotelling's Trace	0.390	0.974	10.000	50.000	0.477
	Roy's Largest Root	0.362	1.953[c]	5.000	27.000	0.118
SAMPLE	Pillai's Trace	1.045	2.994	15.000	84.000	0.001
	Wilks' Lambda	0.205	3.735	15.000	72.176	0.000
	Hotelling's Trace	2.693	4.429	15.000	74.000	0.000
	Roy's Largest Root	2.163	12.114[c]	5.000	28.000	0.000

The means difference was at P≤ 0.05

Appendix XI: Tests between-subjects effects of physico-chemical parameters of the soilusedto grow *Hibiscus sabdariffa*atdifferentconditionsand stages

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
TREATMENTS	N	33755.556	2	16877.778	1.716	0.197
	P	114122.531	2	57061.266	0.357	0.703
	K	1768888.889	2	884444.444	0.996	0.381
	SOIL_PHK	0.053	2	0.027	0.436	0.651
	SOIL_PHW	0.211	2	0.106	2.968	0.067
SAMPLE	N	180211.111	3	60070.370	6.107	0.002
	P	3711307.785	3	1237102.595	7.733	0.001
	K	17063611.111	3	5687870.370	6.404	0.002
	SOIL_PHK	0.382	3	0.127	2.079	0.124
	SOIL_PHW	0.951	3	0.317	8.904	0.000

The means difference was at P≤ 0.05

Appendix XII: Estimated marginal means of physico-chemical parameters of the soil used togrow *Hibiscus sabdariffa*atdifferentconditionsand stages

Dependent Variable	Sample	Mean	Treatments	Mean
N	1ST SAMPLE	396.667±33.059	M	326.667±28.630
	2ND SAMPLE	241.111±33.059	F75	268.333±28.630
	3RD SAMPLE	287.778±33.059	F100	256.667±28.630
	CONTROL	210.000±33.059	M	497.729±115.465
P	1ST SAMPLE	1086.028±133.328	F75	602.729±115.465
	2ND SAMPLE	514.806±133.328	F100	627.667± 115.465
	3RD SAMPLE	506.333±133.328	M	2775.000±272.058
	CONTROL	197.000±133.328	F75	2341.667±272.058
K	1ST SAMPLE	3611.111±314.145	F100	2275.000±272.058
	2ND SAMPLE	2116.667±314.145	M	6.464±0.071
	3RD SAMPLE	2327.778±314.145	F75	6.461±0.071
	CONTROL	1800.000±314.145	F100	6.544±0.071
SOIL_PHK	1ST SAMPLE	6.604±0.083	M	7.178±0.054
	2ND SAMPLE	6.396±0.083	F75	7.001±0.054
	3RD SAMPLE	6.379±0.083	F100	7.037±0.054
	CONTROL	6.580±0.083		
SOIL_PHW	1ST SAMPLE	7.330±0.063		
	2ND SAMPLE	6.952±0.063		
	3RD SAMPLE	6.916±0.063		
	CONTROL	7.090±0.063		

The means difference was at P≤ 0.05

Appendix XIII: **Pearson correlations matrix for proximate, anti-nutrients and soil parameters from the growing stages of *Hibiscus sabdariffa.***

	MC	ASH	CFAT	CP	CF	CARB	CALO	OX	TAN	Ns	Ps	Ks	pH1	pH2
MC	1													
ASH	.049	1												
CFAT	.112	.202	1											
CP	-.022	.579**	.355*	1										
CF	.082	.097	-.267	-.234	1									
CARB	.253	-.102	.062	-.369*	.551**	1								
CALO	.312	.221	.093	.056	.462**	.737**	1							
OX	.127	.232	.021	.212	.044	.406*	.398*	1						
TAN	.151	.080	.154	-.213	.167	.481**	.495**	.063	1					
Ns	.238	.362*	.280	.471**	-.041	-.044	.294	.073	-.017	1				
Ps	-.144	.522**	.185	.456**	.141	-.158	.103	.061	-.088	.294	1			
Ks	.055	.464**	.313	.626**	-.158	-.150	.284	.057	.021	.593**	.597**	1		
pH1	.392*	.381*	.203	.251	.473**	.714**	.912**	.466**	.431**	.358*	.249	.340*	1	
pH2	.482**	.454**	.268	.322	.359*	.644**	.894**	.532**	.411*	.432**	.241	.408*	.969**	1

P≤0.05

Key; MC : moisture contents ,ASH : ash contents ,CFAT: crude fat, CP: crude protein, CF: crude fiber, CARB : carbohydrate ,CALO :calorific value ,OX : oxalate ,TAN : tannin ,Ns : soil nitrogen ,Ps : soil phosphorus, Ks : soil potassium, pH1: pH value in KCl, pH2:pH value in water.

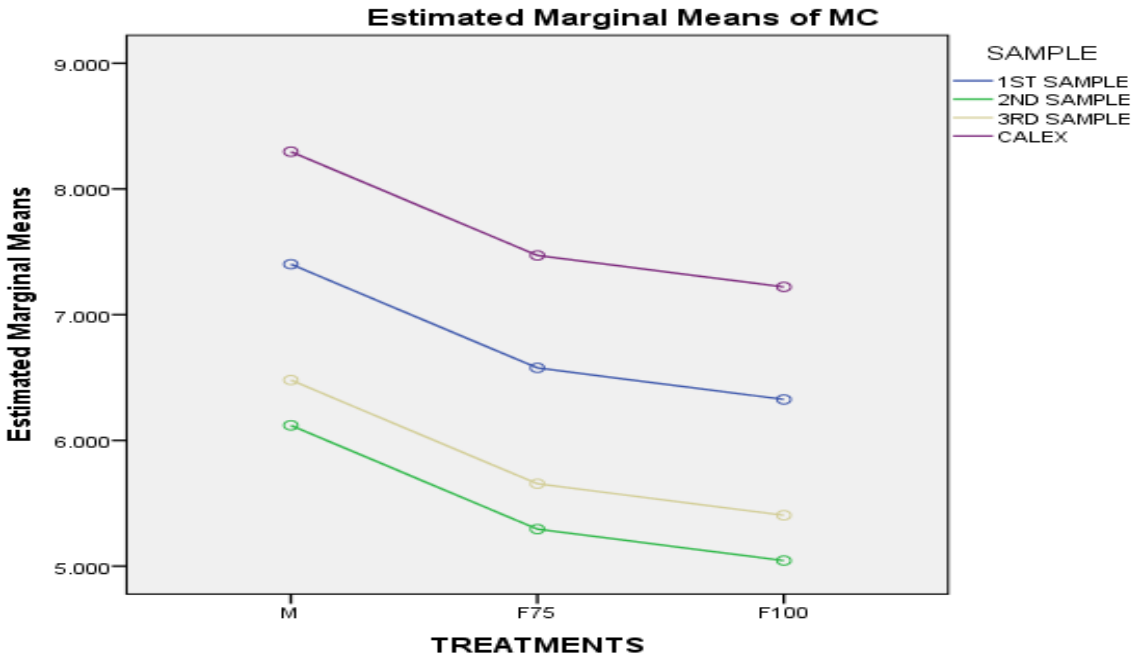
AppendixXIV: Pearson correlations matrix for minerals, anti-nutrientsand soil physico-chemical parameters obtained at the growing stages of *Hibiscus sabdariffa*.

	Na	K	Mg	Ca	Mn	Fe	Zn	Pb	Cu	Ox	Tan	Ns	Ps	Ks	pH1	pH2
Na	1															
K	-.067	1														
Mg	.325	-.152	1													
Ca	.153	-.363*	.261	1												
Mn	.015	.595**	.155	.410*	1											
Fe	.316	-.320	.001	.143	.349*	1										
Zn	.102	.731**	-.029	.326	.534**	-.329*	1									
Pb	.446**	.199	-.383*	.019	-.052	.458**	.232	1								
Cu	.483**	-.338*	.573**	.417*	.360*	.475**	.348*	.638**	1							
Ox	.173	-.030	.129	.146	.227	-.025	.112	-.102	.034	1						
Tan	-.238	.310	-.246	.093	-.367*	-.378*	.165	.282	-.318	.200	1					
Ns	.298	-.022	.299	.212	.084	.350*	.343*	.568**	.622**	.133	.215	1				
Ps	.458**	-.371*	.296	.164	.059	.145	-.232	.542**	.356*	.038	.196	.246	1			
Ks	.674**	-.069	.509**	.126	.006	.243	-.152	.564**	.488**	.135	.154	.536**	.577**	1		
pH1	.237	.081	.025	.092	-.341*	.016	.184	-.168	.045	.069	.006	.013	.283	.045	1	
pH2	.555**	.214	.372*	.028	-.199	.205	.201	.614**	.436**	.274	.125	.385*	.267	.386*	.367*	1

$P \leq 0.05$

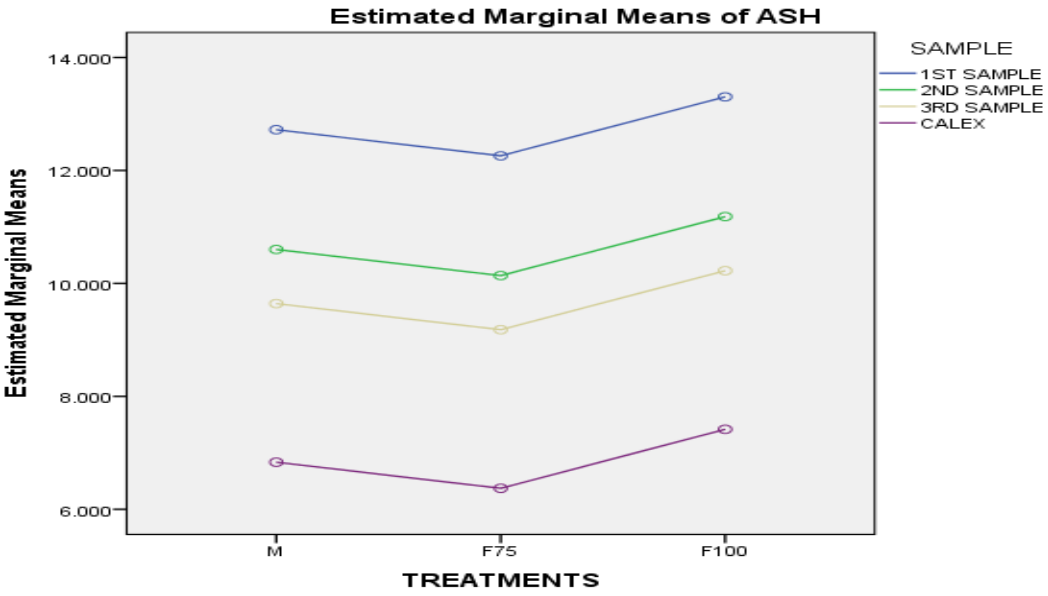
OX: oxalate ,TAN : tannin ,Ns : soil nitrogen ,Ps : soil phosphorus, Ks : soil potassium, pH1: pH value in KCl, pH2:pH value in water.

(%)



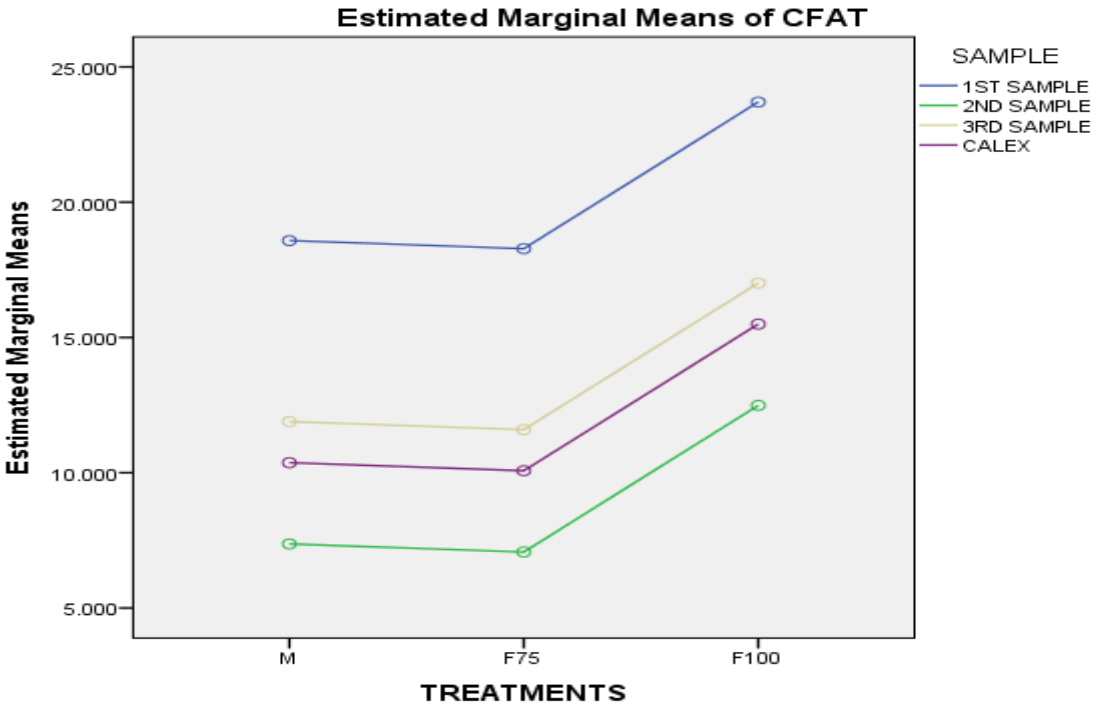
Appendix IV: Moisture contents for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(%)



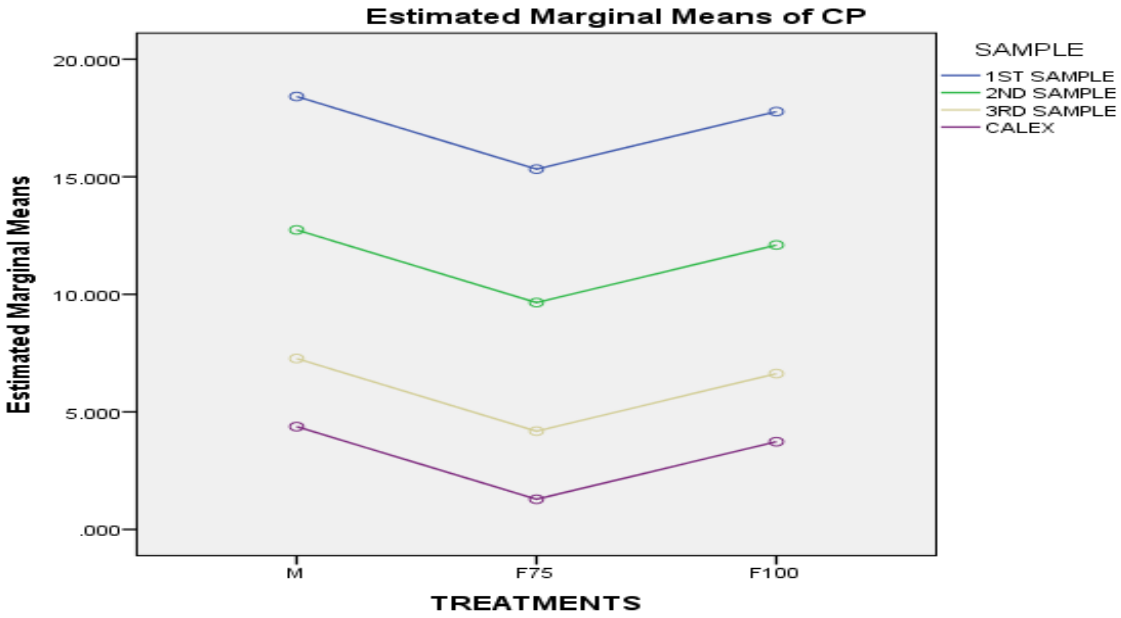
Appendix XVI: Ash content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(%)



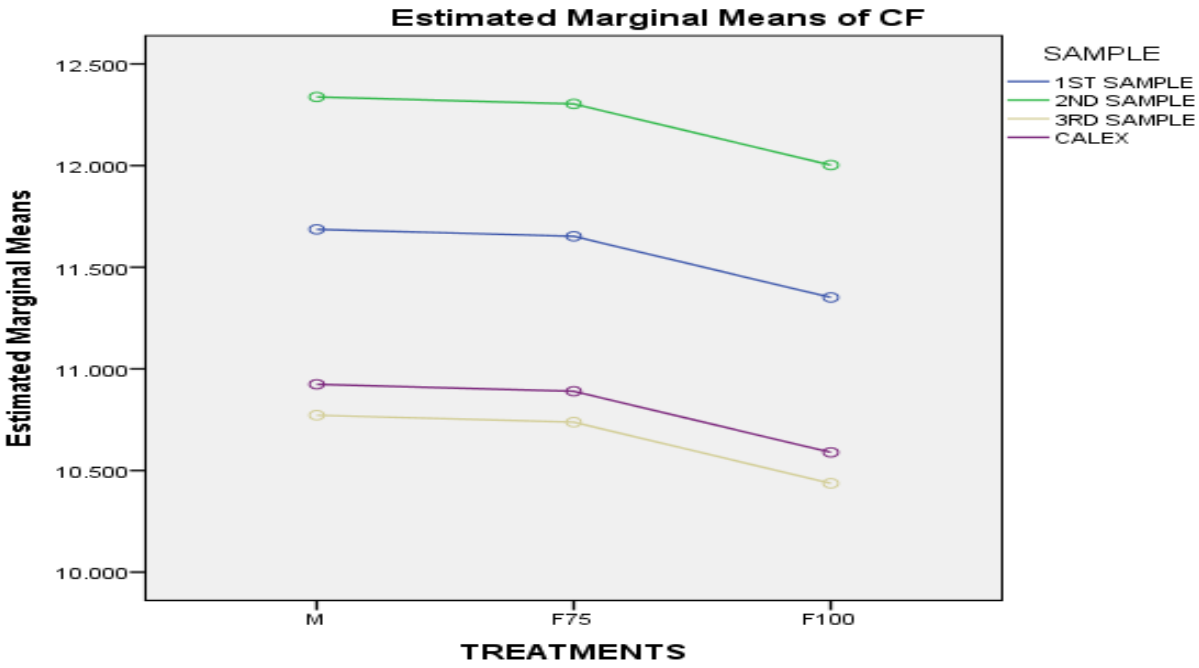
Appendix XVI: Crude fat content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(%)



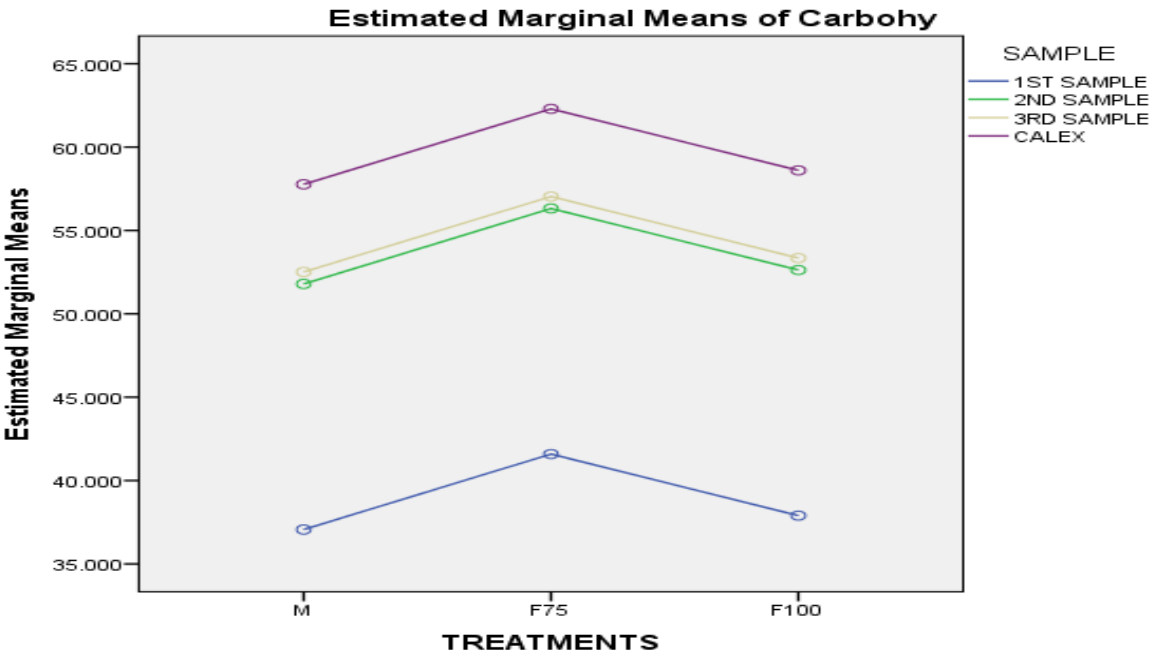
Appendix XVII: Crude protein content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(%)



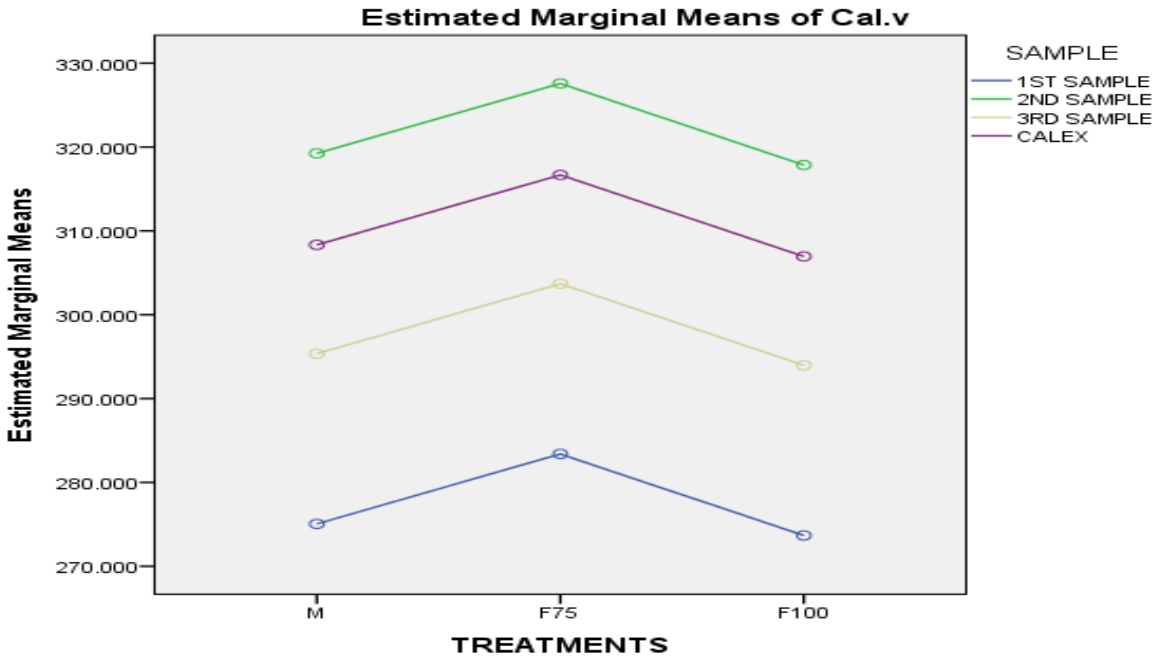
Appendix XIX: Crude fiber content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(%)



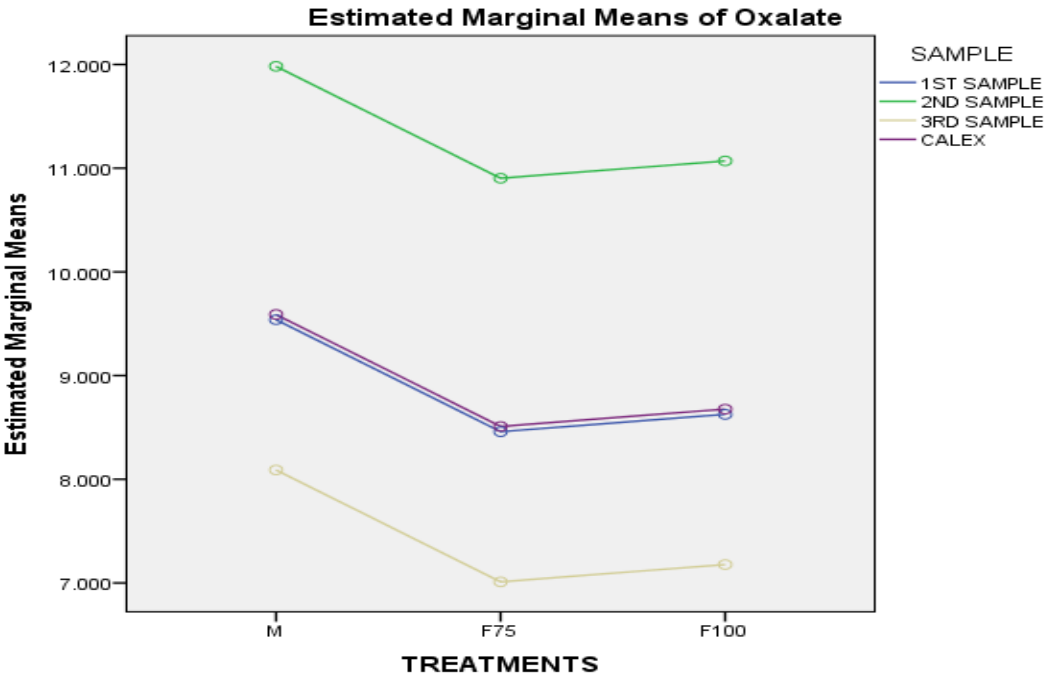
Appendix VIII: Carbohydrate content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(kcal)



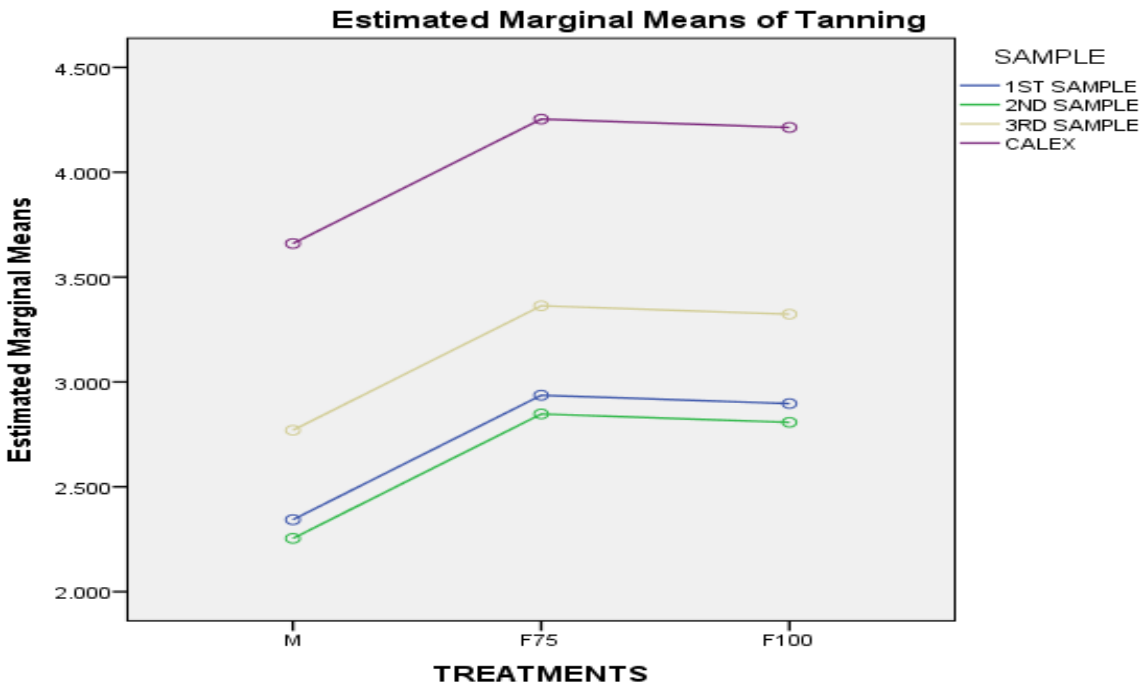
Appendix IXXI: Calorific value content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(%)



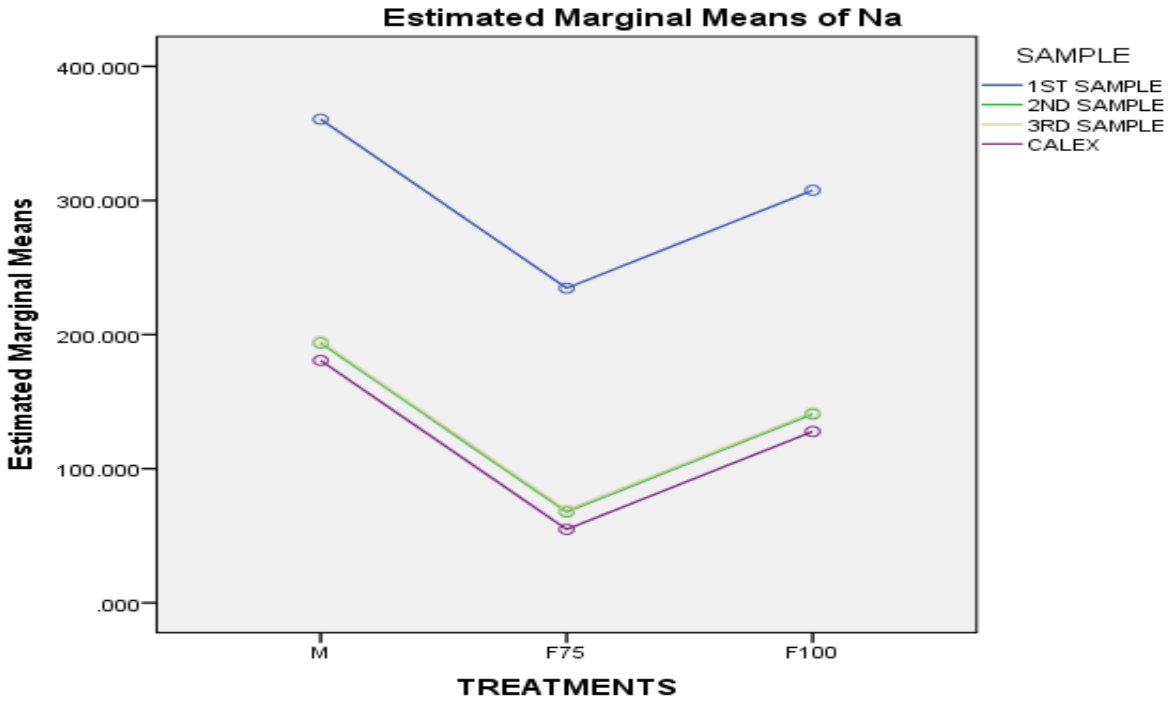
Appendix X; Oxalate content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(%)



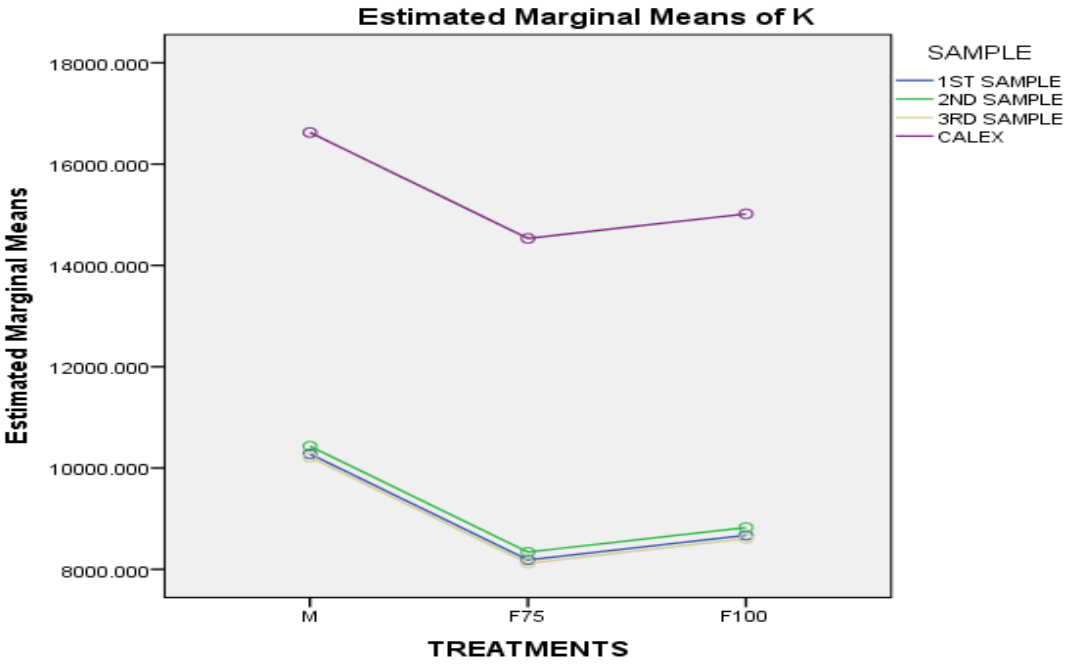
Appendix XI; Tannin content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(mg/kg)



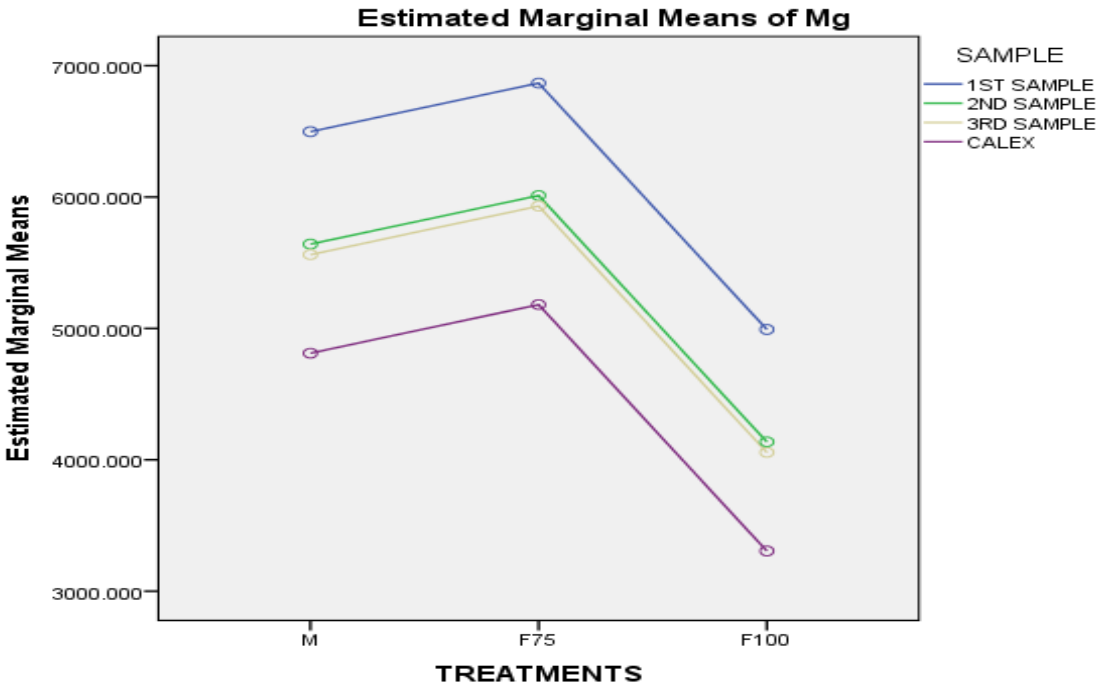
Appendix XIIV: Na for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(mg/kg)



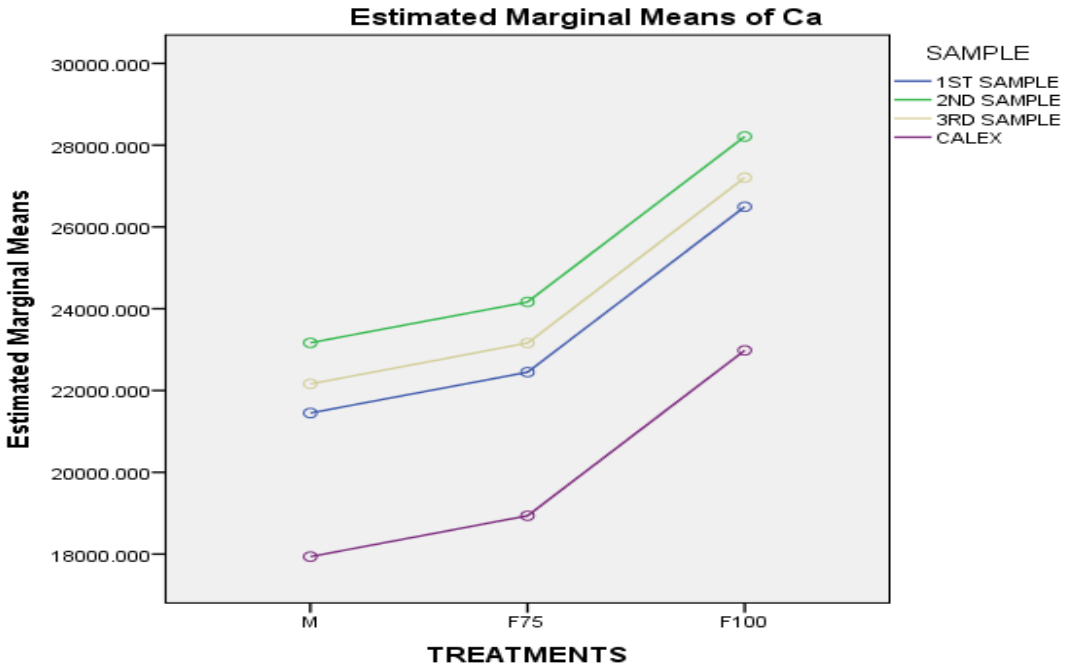
Appendix XIII: K for *H. sabdarifa* leaves and calyx from the levels of fertilizer used at the growing stages

(mg/kg)



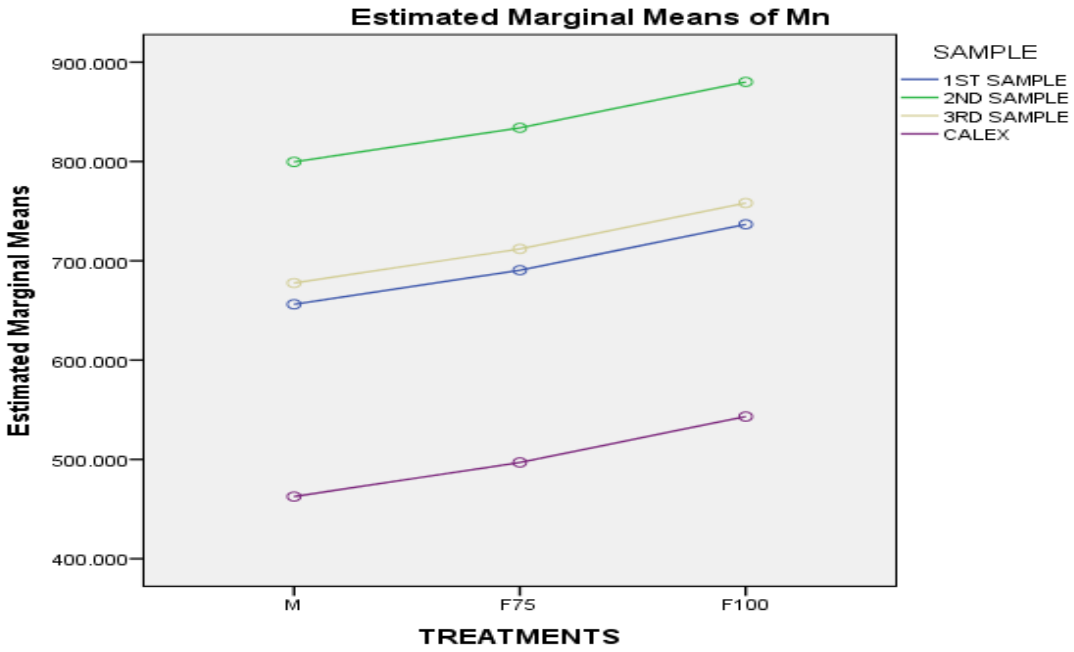
Appendix XIVI: Profile Plots of Mg content for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(mg/kg)



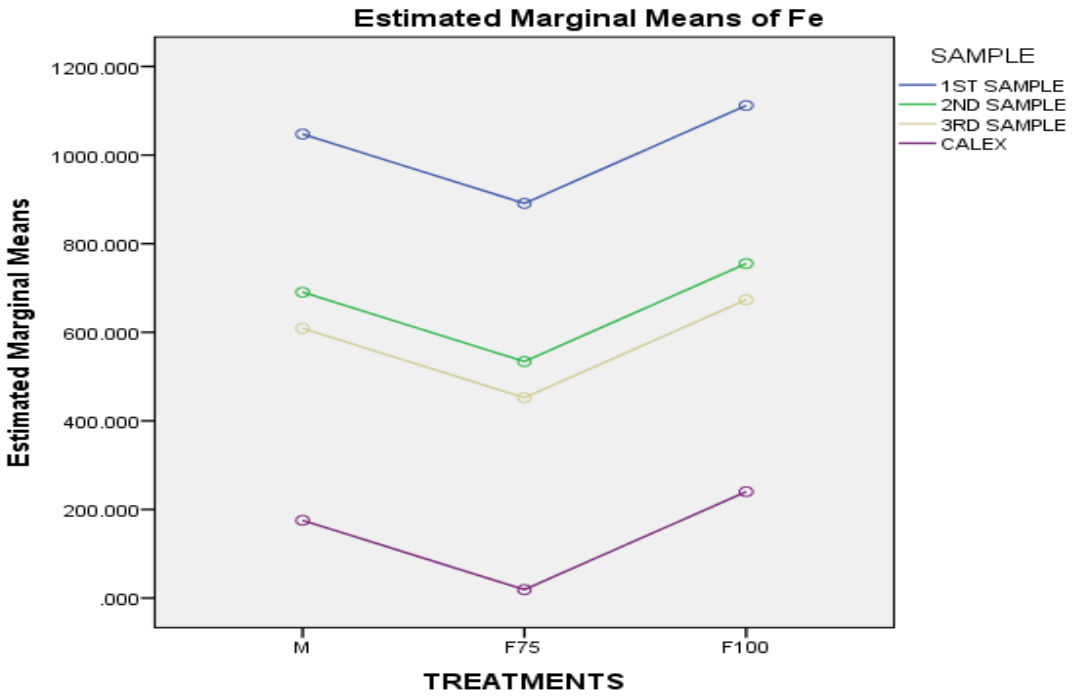
Appendix XVI: Ca for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(mg/kg)



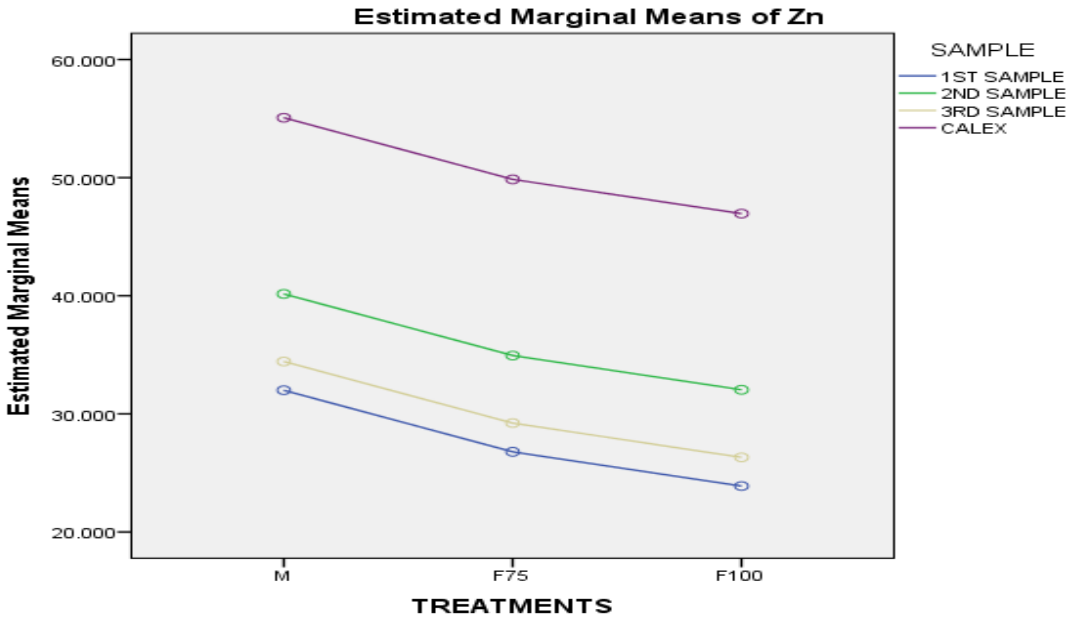
Appendix XVI: Mn for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(mg/kg)



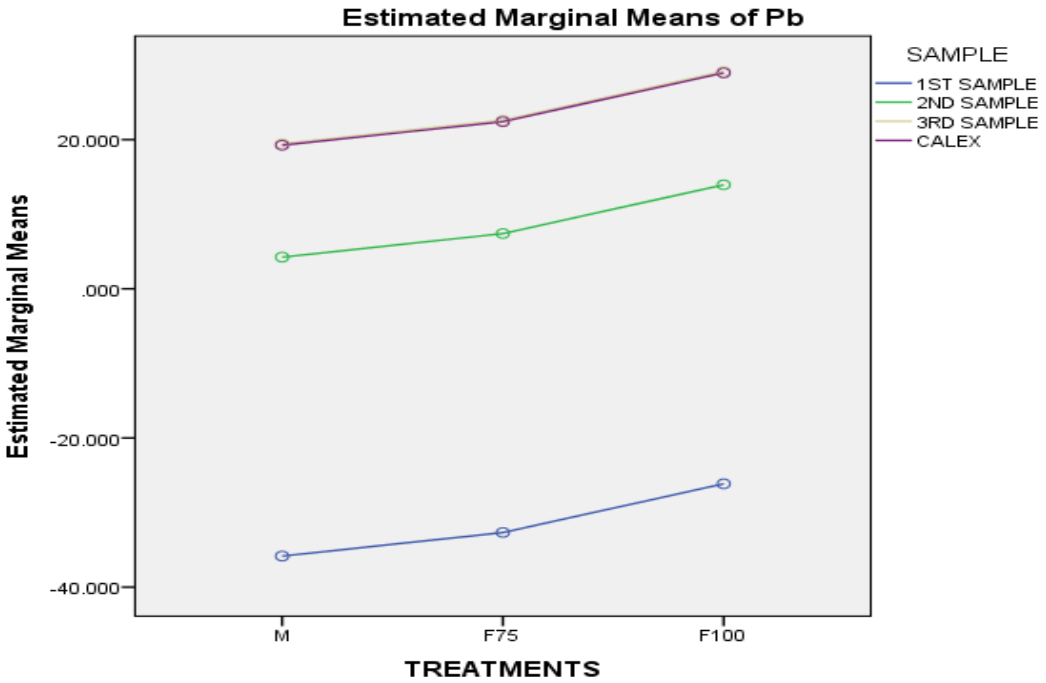
Appendix XVIIX: Fe for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(mg/Kg)



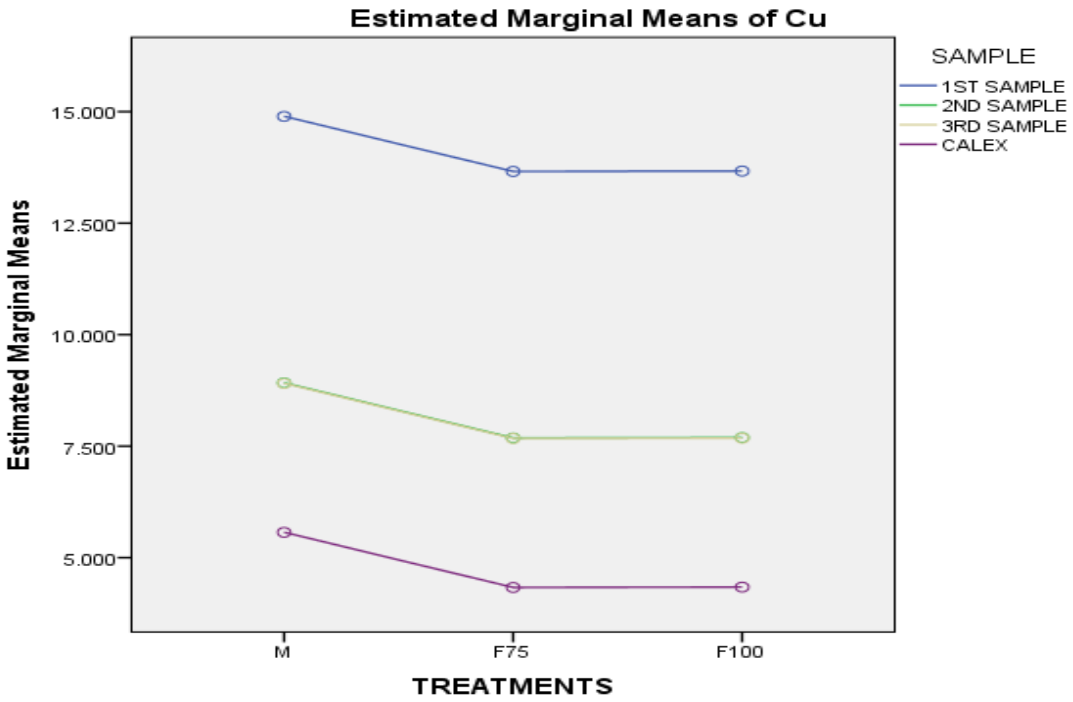
Appendix XVIII: Zn for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(mg/kg)



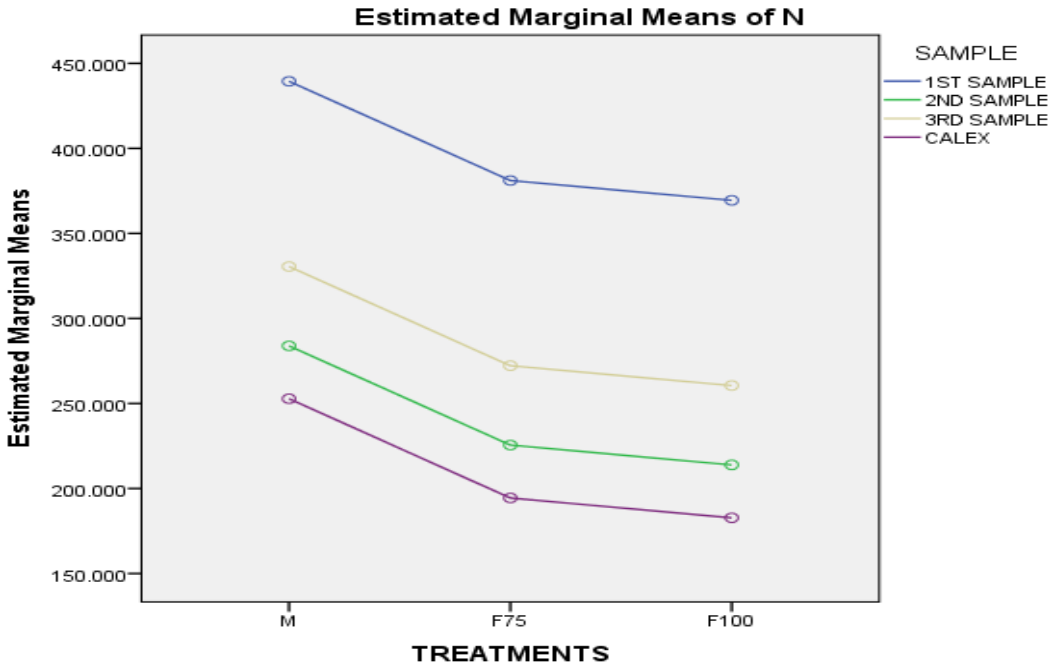
Appendix XXXI: Pb for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(mg/kg)



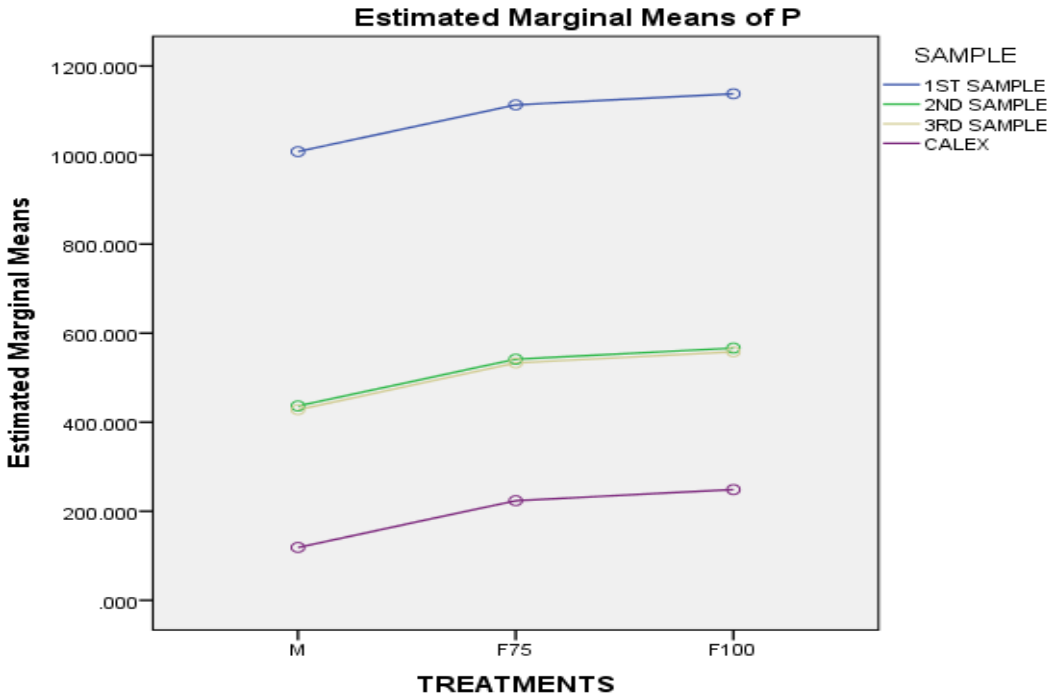
Appendix XIXXII: Cu for *H. sabdariffa* leaves and calyx from the levels of fertilizer used at the growing stages

(mg/kg)



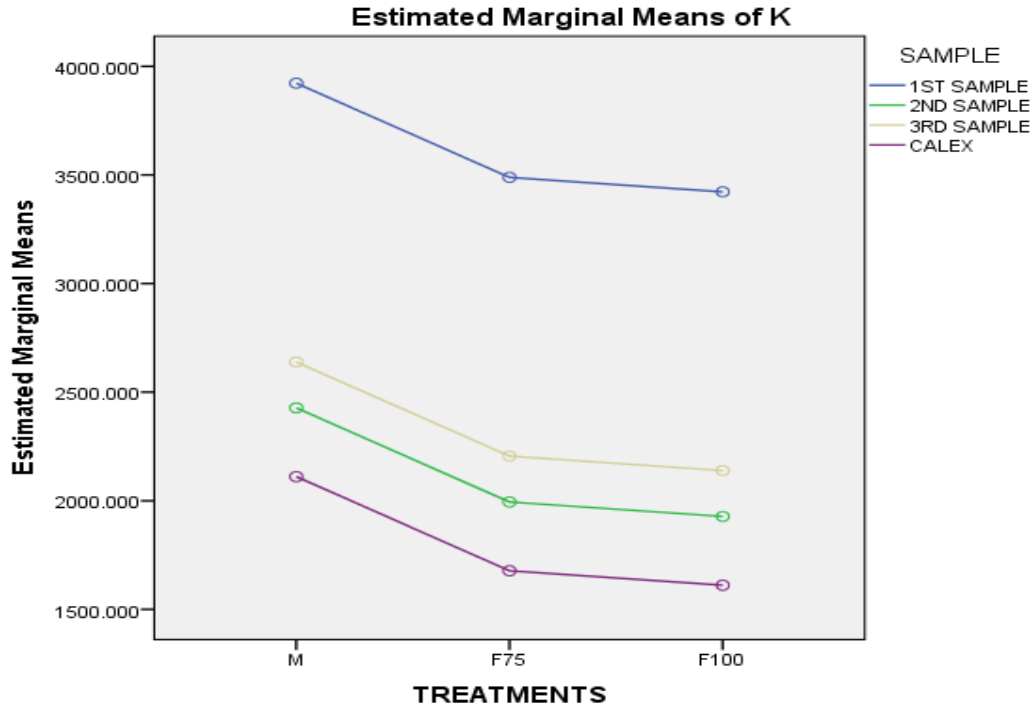
Appendix XX: Soil N from the levels of fertilizer used at the growing stages

(mg/kg)



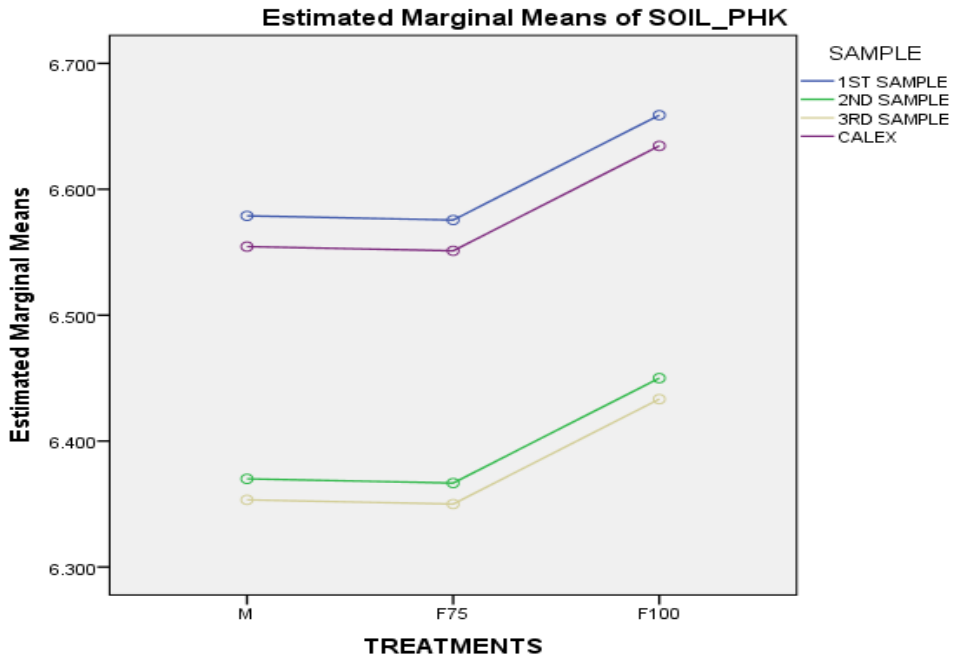
Appendix XXIV: Soil P from the levels of fertilizer used at the growing stages

(mg/kg)



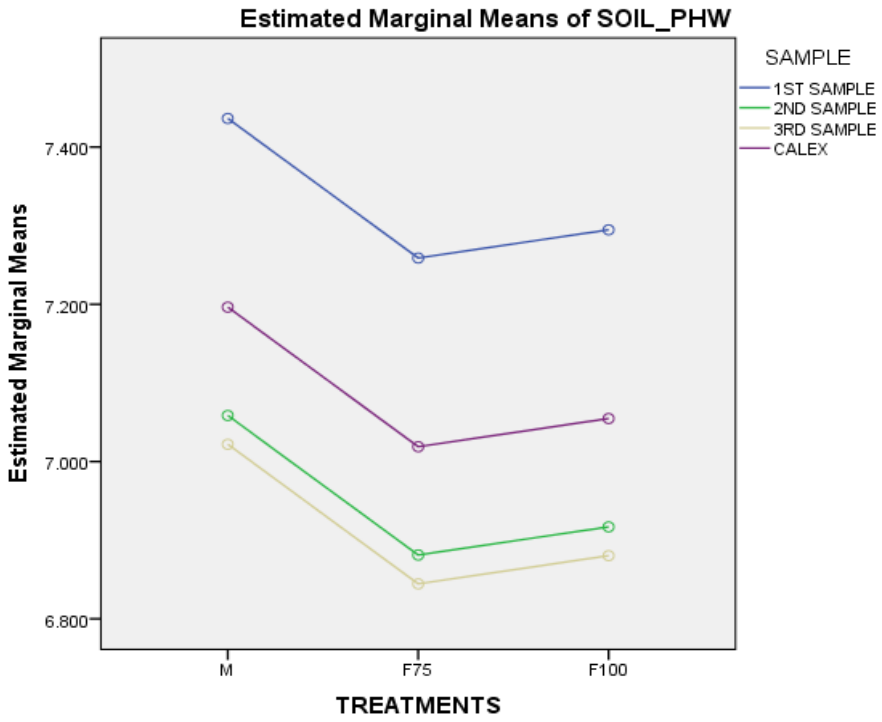
Appendix XXIIV: Soil K from the levels of fertilizer used at the growing stages

pH level



Appendix XXIIII: Soil pH in KCl from the levels of fertilizer used at the growing stages

pH level



Appendix XXIVII: Soil pH in water from the levels of fertilizer used at the growing stages







Appendix XXV: Vegetative Stage (SI) of *Hibiscus sabdariffa*





Appendix XXVIX: Flowering stage (SII) of *Hibiscus sabdariffa*





Appendix XXVIIXX: Maturity /Fruiting Stage (SIII)of *Hibiscus sabdariffa*



Appendix XXVIIII: A dried Calyx (fruit)