ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
CENTER FOR FOOD SCIENCE AND NUTRITION

Possible Consequences of Atmospheric CO_2 Level Increment on Chemical Composition and Functional Properties of Maize (*Zea mays* L.) and Wheat (*Triticum aestivum* L.) Varieties in the Past Thirty Years Collected From Ambo District, Amaro Kebele, West Shoa Zone of Oromia, Ethiopia

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List of Acronyms

BD	Bulk Density
Bt	Billion Tons
C_3	Three-Carbon Organic Acids
C_4	Four-Carbon Organic Acids
CAM	Crassulacean Acid Metabolism
CFC	Chloro-Fluoro-Carbon
CH_4	Methane
CO_2	Carbondioxide
CO	Carbonmonoxide
CO_2e	Carbondioxide Equivalent
FACE	Free Air Carbon Enrichment
G.C	Gregorian Calendar
Gg	Giga Gram (1 million gram; 1000 kg, or 1 tone)
Mt	Metric Tone
GHGs	Green House Gases
Gt	Giga Tonnes (10^9 Mt)
hr	Hour
IPCC	Intergovernmental Panel on Climate Change
IR	Infrared Radiation
MEF	Ministry of Environment and Forest
N	Nitrogen
N_2O	Nitrous Oxide
O_3	Ozone
^{O}C	Degree Celsius
OAC	Oil Absorption Capacity
OTC	Open Top Chambers
ppm	Parts Per Million
Rubisco	Ribulose Bisphosphate Carboxylase-Oxygenase
UNFCCC	United Nations Framework Convention on Climate Change
WAC	Water Absorption Capacity
WHO	World Health Organization

ABSTRACT

Human activities contribute to climate change by causing changes in earth's atmosphere in the amounts of Green House Gases. Increment in atmospheric carbondioxide due to different anthropogenic activities are affecting yield of crops by increasing the rate of photosynthesis. This condition frequently alters chemical compositions of crops as a result of increase concentration of carbon in their tissues, with correspondingly reduced concentrations of other essential elements like protein and minerals. Based on this fact, this study was conducted in order to evaluate the possible consequences of increasing atmospheric carbondioxide level on chemical composition and functional properties of bread wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) from the past thirty years with five year intervals. The samples were collected from gene bank of Ethiopian biodiversity institute, which was collected from Ambo district, West Shoa Zone of Oromia. The official methods used were AOAC (2000, 2010, 2016), Elena (2012), AOAC (2001) and (Sathe *et al*., 1982a), (Adeleke *et al*., 2010), (Maninder *et al.*, 2007) for Proximate composition, mineral analysis, total glucose and functional properties analysis respectively. Both samples with two different carbon fixation photosynthetic pathways show a significant ($p<0.05$) increment in moisture, utilizable carbohydrate and total glucose in the past three decades. Crude fat, crude protein contents shows a decrement ($P<0.05$) in *Zea mays* L. and *Triticum aestivum* L. (8.40-6.07%, 6.25% - 4.07%) and (3.70% –1.43% and 14.13–8.58%) respectively from 1987 to 2017 G.C. All minerals contents analyzed in *Zea mays* L. and *Triticum aestivum* L. flour sample have shown reduction at ($P<0.05$) with values of (22.05-15.97, 120.10-110.01, 2.02-1.98 and 1.99-1.87) and (31.5-29.09, 70.01-60.96, 1.49–0.95 and 2.20-1.03 mg/100gm) of Ca, Mg, Zn and Fe for both samples, respectively. The functional properties of the two samples were also investigated. The Water absorption capacity and bulk density for *Zea mays* L. and *Triticum aestivum* L. were recorded with significant increment at ($P<0.05$) and oil absorption capacity values on the other hand significantly reduced from (1.76-1.43 and 1.95-1.27 ml/g), respectively for each samples. Wet gluten content of *Triticum aestivum* L. also analyzed and show a decrement at ($P<0.05$) from (23.92% to 22.40%) in the past three decades. It can be concluded that direct effect of carbondioxide "fertilization" on the two key crops samples in this study has increase carbohydrate production and lower the levels of fat, protein, essential minerals and functional properties except water absorption and bulk density. Results in this study also show that three carbon photosynthetic pathway crop *Triticum aestivum* L. shows a notable reduction especially in protein content, Ca, Zn, Fe, wet gluten and increment in carbohydrate and total glucose content than that of four carbon photosynthetic pathway representing crop *Zea mays* L. within five years intervals in the past three decades which will be a large burden to nutritional perspective. And will be able to lead serious consequences of deficiencies and diseases for the future as this greenhouse gas in the atmosphere continues to increase.

Key words: Green house gases, *Zea mays* L., *Triticum aestivum* L., Carbondioxide, Carbondioxide "fertilization"

CHAPTER ONE

1. Introduction

1.1 Background of the study

Parts of the earth’s atmosphere act as an insulating blanket of just the right thickness, trapping sufficient solar energy to keep the global average temperature in a suitable range and this blanket is a collection of atmospheric gases called Green House Gases (GHGs) based on the idea that the gases also 'trap' heat like the glass walls of a greenhouse (IPCC, 2007b). The global average surface temperature increased by 1.5 - 4.5 ^{0}C over the next 100 years, which is primarily caused by the building up of GHGs in the atmosphere and global warming, is a specific example of the broader term “Climate Change” (Vimal *et al*, 2017).

There are principal Green House Gases (GHGs) emissions due to different human activities, they absorbs energy which radiates from the surface or atmosphere of the earth and these gases which act as effective global insulators, reflecting back to earth visible light and infrared radiation. CO_2, CH_4, N_2O, O_3, CFC, and halocarbons are the common GHGs able to accumulate in the atmosphere, significant increases in all of these gases have occurred in the industrial era (Ahmad and Nour, 2015). Impact of global environmental change on agriculture has a multitude of aspects and the two most important changes in atmospheric composition are the rising of CO_2 and reducing in O_3 concentrations (Johan and Kan, 2012).

Carbondioxide (CO_2) is a minor constituent of the atmosphere but the most important GHG and climatic changes in the past have been associated with changes in the atmospheric gas (Ahmad and Nour, 2015). The effects of global warming on food production are complex, and are a combination of increasing CO_2 concentrations in the atmosphere, higher temperatures, and fluctuations in rainfall (Peshev *et al*., 2014).

Studies have shown that higher concentration of atmospheric CO_2 affect crops in two important ways: they boost crop yields by increasing the rate of photosynthesis which increases growth, and they reduce the amount of water crops lose via transpiration (Nereu, 2005). Crop production will be affected by global warming, resulting in world-wide food shortages and starvation. Increased concentrations of CO_2, one of the main substances responsible for global warming, will promote plant growth through intensified photosynthesis and rise in the levels of CO_2 would actually benefit plants rather than harm those (Masahumi *et al*., 2011).

On increased atmospheric CO_2 some crop plants most exhibit reduces on the stomatal openings, which may leads to reduction of transpiration per unit leaf area while enhancing photosynthesis and this may tends to increase growth and yield of most agricultural plants (Parrya *et al*., 2004). The 'fertilizing effect' of elevating CO_2 concentrations on crop yields will decline slightly because of the negative effects of rising temperature and other factors relate to the increment of this GHG (Loladze, 2002).

Numerous effects of elevating atmospheric CO_2 level on plants have been documented, including increasing concentrations of this gas; lead plants show increased concentrations of carbon with reduced concentrations of other elements their tissues (Gifford *et al*., 2000). Eventhough, wheat, rice, maize and soybeans and other common grains are relatively low in iron and zinc, in Africa and other poorer societies where meat and meat product is rarely eaten they are a major source of the macro and micro nutrients and about 2.4 billion people currently get at least 60% of their zinc and over 75% their iron from these staples grains (Stoltzfus *et al*., 2004).

Ethiopia is an agrarian economy based country largely depends on agricultural sector to meet domestic food demand economy; it contributes to nearly 80% of export earnings and provides employment to more than 73% of the population (EATA, 2014). In Ethiopia the annual minimum temperature over the country has been increasing by about 0.25 ^{0}C every 10 years, while annual maximum temperature has been increasing by about 0.1 ^{0}C in every decade (NMSA, 2001).

Agricultural crops are common sources of dietary nutrients including zinc, iron, magnesium, phosphorus in Ethiopia and rising CO_2 emissions are set to make these staple food crops less nutritious, it is appropriate to use this as a background to investigate the possible effects of atmospheric CO_2 which is the major contributor to climate change. Therefore this thesis was studied the possible consequences of increasing atmospheric CO_2 level on chemical composition and functional properties of maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) varieties which are major dominant cereal crops with two different carbon fixation photosynthetic pathways (C_4 and C_3), respectively. The samples collected from Ambo District, West Shoa Zone of Oromia and accounted three past decades. Ambo classified under sub-tropical agro-ecological zone and 114 km away west of Addis Ababa lying between 80 47' - 90 21' North latitude and 37 32' - 38 3' East longitude with sandy loam and clay soil type (Ambo District Agriculture and rural development office report, 2014).

1.2. Statement of the problem

Elevating level of atmospheric CO_2 has a significant effect on crop yield production on a global scale. In fact, scientific publication have produced a valuable information based on controlled environment on the exceptional effects of elevating CO_2, temperature rise and water supply on crop growth and yield, which critically increasing understanding of the dynamics of photosynthesis, biomass accumulation and crop yield that are necessary to forecast impacts of climate change on agriculture (Tubiello *et al*., 2007).

Crop quality is thought to be a multi-sided and complex subject involving growth, pre and post-harvest, storage and including nutritional, technological and environmental facets (Hay and Porter, 2006). Not only tissue nutrient concentrations but also the nutritional value of crop’s grains are also expected to be affected by alterations in transpiration under high atmospheric CO_2 (Fabio *et al*., 2010).

Since CO_2 is the largest contributor to the change in climate and one of the many ways that climate change may affect human health is by altering the nutrient content of crops grains and this issue have been limited by artificial growing conditions like Open Top Chamber (OTC) and Free Air Carbondioxide Enrichment (FACE) than field trials examination which have the potential to provide results that will be more realistic (Johan and Kan, 2012), only in foreign countries.

Due to the increment of atmospheric CO_2 decreases in essential elements in grains of legumes and non-legumes such as wheat, barley, maize, soybean, sorghum and rice are expected, which ultimately will aggravate and putting millions at risk of the ‘‘hidden hunger” of micronutrient malnutrition in the world. The other issue is that as atmospheric CO_2 rising it revs up photosynthesis, the process that helps crop plants transform the sunlight to food and this makes them to grow faster, but it also leads them to pack in more carbohydrates like glucose at the expense of other nutrients that most population across the world depend on which is able to lead deficiencies and other diet related diseases such as diabetes, heart disease and stroke and also obesity (Loladze, 2002).

Eventhough it’s reported by different publications that the impact of increasing atmospheric CO_2 level on over all nutritional compositions of crop grains and the there was no study done before on this area in our country, the present study attempt to investigate and put an effort on this hidden issue which is leading us to have insufficient knowledge in order to draw firm conclusions on how the past, current and ongoing changes of this GHG affect crops quality for human nutrition. The current practices prevail, that CO_2 emissions in Ethiopia will be more than double from 150 Mt CO_2 to 400 Mt CO_2 in 2030 and reaches to 1000 ppm globally at the end of this century.

Therefore, based on this fact, this thesis was studied the possible consequence of increasing atmospheric CO_2 level on chemical composition and functional properties of bread wheat (*Triticum aestivum* L.) and white maize (*Zea mays* L.) which are the most important cereals cultivated and common staple food source in Ethiopia and ranks as second and fourth in coverage area and production (CSA, 2012) and represent different response to increasing atmospheric CO_2 because of their different Carbon fixation photosynthetical pathways, harvested from Ambo District, Amaro kebele, West Shoa Zone of Oromia region, in past three decades from (1987 G.C to 2017 G.C).

1.3. Objectives

1.3.1. General Objective

To evaluate possible consequence of increasing atmospheric CO_2 level on chemical composition and functional properties of maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) from last three decades.

1.3.2. Specific Objectives

The specific objectives of this study were to:-

- ✓ Analyze possible effect of increasing atmospheric CO_2 on carbohydrate and mineral content (Ca, Mg, Zn and Fe) of *Zea mays* L. and *Triticum aestivum* L. collected from Ambo district, Amaro kebele, West Shoa Zone of Oromia.

- ✓ Analyze the effect of elevating atmospheric CO_2 on functional properties (Water absorption capacity, Oil absorption capacity, Bulk Density and Wet gluten) of *Triticum aestivum* L. and *Zea mays* L. in the past three decades with five years interval.
- ✓ Understand effect of elevating atmospheric CO_2 on total glucose content of *Triticum aestivum* L. and *Zea mays* L. in the past three decades.
- ✓ Correlate the values of utilizable carbohydrate with protein content and also mineral contents (Ca, Mg, Zn and Fe) of the two samples with different carbon fixation photosynthetic pathways, C_3 (*Triticum aestivum* L.) and C_4 (*Zea mays* L.) in the past three decades.

1.4. Significance of the study

- ✓ Most of all the result of this research would reveal and significantly help in understanding the possible effect and relation of increasing atmospheric CO_2 with chemical composition and functional properties of grains.
- ✓ Encourage policymakers and concerned bodies to discuss strategies to minimize impacts of increasing atmospheric CO_2 on nutritional quality of cereals which are the major food source of our country.
- ✓ Students, teachers, researchers and academicians of the field area could use the findings of the study as a reference material.
- ✓ This thesis would also use for future research themes to continue on search of alternatives to deal with increasing atmospheric CO_2 and to give the next gap to be covered as recommendation.

CHAPTER TWO

2. Literature Review

2.1. Climate change

Climate change in Intergovernmental Panel on Climate Change (IPCC) usage refers to “a change in the state of the climate that can be identified (using statistical tests) by changes in the mean and/or the variability of its properties that persists for an extended period, typically decades or longer. It refers to any change in climate over time, whether due to natural variability or as a result of human activities” (IPCC, 2007a).

Past climate changes led to extinction of many plant and animal species, population migrations, and pronounced changes in the land surface and ocean circulation. The speed of the current climate change is faster than most of the past events, making it more difficult for human societies and the natural world to adapt (IPCC, 2013). Climate changes are assumed to have effects through GHGs effect, rainfall, soil moisture, temperature and radiation variability effects (Leff *et al*., 2004).

It will amplify existing risks and create new risks for natural and human systems. Risks are unevenly distributed and are generally greater for disadvantaged people and communities in countries at all levels of development. Many aspects of climate change and associated impacts will continue for centuries, even if anthropogenic emissions of GHGs are stopped (IPCC, 2001).

Climate change is not only something of the future, but the climate has already changed significantly over the last 30 years (Fulco *et al*., 2007). It have a high negative impact and it will make problems worse which are related to rapid population growth, existing poverty and a heavy reliance on agriculture and the environment (Revati *et al*., 2015).

However developing countries will suffer the most from climate change because of the economic importance of climate sensitive sectors such as agriculture in combination with their low adaptive capacity and they have a much more limited capacity like lack human and financial capacity to deal effectively with the problems caused by climate change (IPCC, 2007b).

Due to increment of GHGs, the atmospheric temperature is increasing and this also affect agriculture and natural ecosystems either directly via changes in agro-ecological conditions or indirectly via changes in economic growth and distribution of incomes, which in turn affect demand for agricultural produce (Aggarwal, 2003). Anthropogenic GHGs emissions have increased since the pre-industrial era, driven largely by economic and population growth, and are now higher than ever. This has led to atmospheric concentrations of CO_2, CH_4 and N_2O; those are unprecedented in at least the last 800,000 years. Their effects, together with those of other anthropogenic drivers, have been detected throughout the climate system and are extremely likely to have been the dominant cause of the observed warming since the mid-20th century (IPCC 2001).

Malnutrition is viewed as one of the common adverse health impacts of climate change and above 25 million children less than 5 years will be at risk of malnutrition by 2050 because of this climate changes (IPCC, 2014). Climate change is likely to reduce agricultural production and affect more than 30% of the farmers in developing countries that already are food insecure. Changes in weather averages, climate variability, and extreme weather events particularly floods and droughts determine the quantity, quality, and stability of crop yields (Porter and Semenov, 2005).

Africa has been identified as one of the parts of the world most vulnerable to the impacts of climate change (IPCC, 2014). Climate change will affect the quality of food crops as well as their quantity. Recent evidence confirms that increasing atmospheric CO_2 significantly decreases the concentrations of zinc, iron, calcium, phosphorus and proteins in wheat, barley, maize and rice which are the main staple African countries (Revati *et al*., 2015).

2.2. Greenhouse Gases

The “Greenhouse Effect” is a term that refers to a physical property of the earth's atmosphere. If the earth had no atmosphere, its average surface temperature would be very low of about -18 ^{0}C rather than the comfortable 15 ^{0}C found today. Difference in temperature is due to a suite of gases called GHGs which affect the overall energy balance of the earth's system by absorbing infrared radiation (Anil *et al*., 2015).

The greenhouse effect can also be compared to the inside of a car parked in sunlight, which lets the light in, but traps the heat and energy, keeping the car warm. The greenhouse effect is very essential, without which the earth would not be warm enough for humans to survive (Ahmad and

Nour, 2015). In its existing state, the earth atmosphere system balances absorption of solar radiation by emission of infrared radiation to space (Figure.2.1). Incoming solar energy called solar radiation warms the earth and the warmed earth radiates heat. However, this is not called ‘heat’, but rather in scientific terms it is IR; atmospheric blanket contain gas molecules (GHGs) those are able to absorb IR leaving the surface and gases are energized, and then emit more IR. Some of this IR returns to the earth surface, warming it further (Boysen *et al.*, 2014, Anil *et al.*, 2015).

Due to GHGs, the atmosphere absorbs more infrared energy than it re-radiates to space, resulting in a net warming of the earth atmosphere system and this is the “Natural Greenhouse Effect”. With more GHGs released to the atmosphere due to different human activities, more IR will be trapped in the earth's surface which contributes to the “Enhanced Greenhouse Effect”.

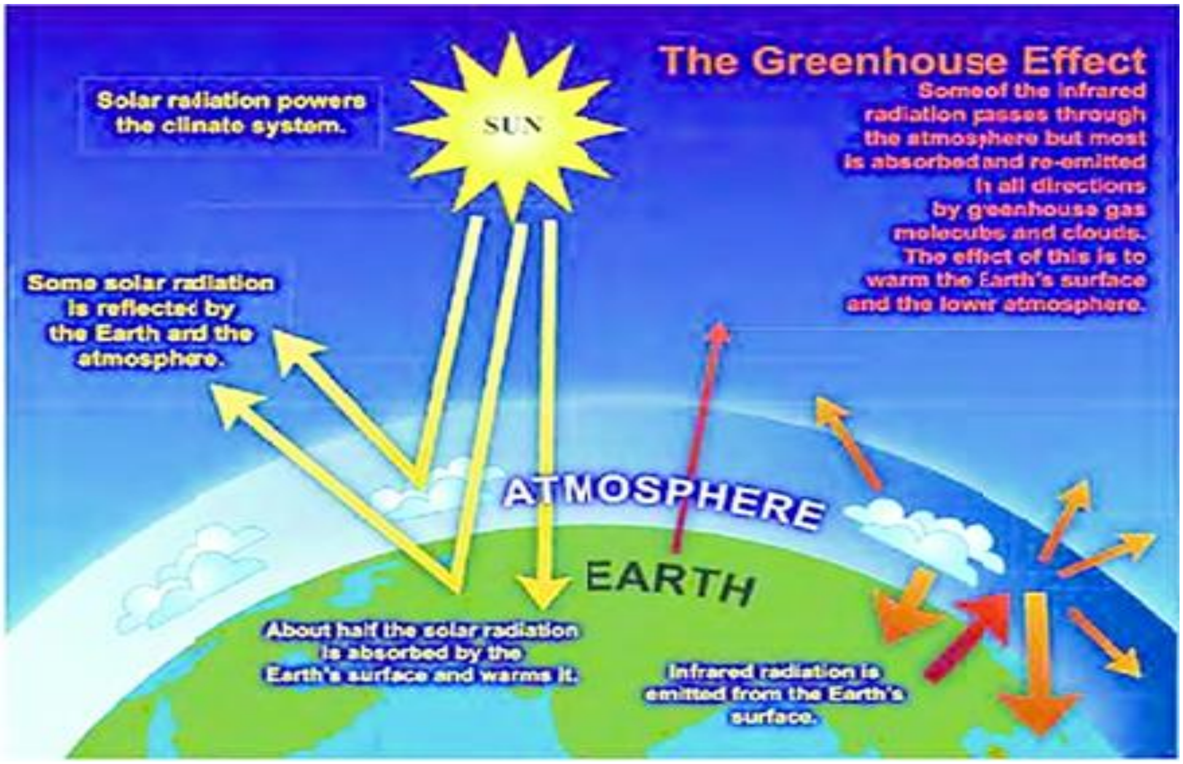

Figure: 2.1. Simplified diagram illustrating the global radiative balance of the atmosphere (Source: IPPC, 2007; Anil *et al.*, 2015)

GHGs are gases in the earth’s atmosphere those allow solar energy to pass through to the earth’s surface. They are able to absorb energy radiated back from the earth and re-radiate that energy back to the surface, thereby making the earth's surface warmer. Due to increased levels of GHGs the atmospheric temperature is increasing and this also affects both agriculture and natural ecosystems, because of increment of these gases current global heat balance is thus upset and leads to a warming of earth's surface (The Causes of Global Climate Change, 2006).

Rise in environmental temperature and changes in related processes are directly connected to increasing anthropogenic GHGs emissions in the atmosphere (Bjorn *et al*., 2007). From the most common causes of climate change, changes in land use is the major one especially in developing countries which include changes in the way people use land for farms, cities and others can lead to warming effects by changing the reflectivity of earth's surfaces (IPCC, 2007a, Boysen *et al*., 2014).

Emissions of pollutants is also the other cause of climate change from industrial, diesel vehicles, diesel engines, coal and biomass stoves and waste incineration, emit pollutants that produce aerosols (small droplets or particles suspended in the atmosphere). Most aerosols cool earth by reflecting sunlight back to space. Black carbon particles (or 'soot') produced when fossil fuels or vegetation are burned, generally have a warming effect because they absorb incoming solar radiation (The Causes of Global Climate Change, 2006).

2.2.1. Principal Greenhouse Gases on climate change

The Initial National Communication of Ethiopia to the UNFCCC submitted to the Convention Secretariat (NMA, 2001) documented that globally the energy sector is the biggest contributor to the emission of greenhouse gases (GHGs). International aviation emissions are also have an effect on GHGs emissions and expected to grow from 0.5 $GtCO_2e$ in 2017 to around 1.1 $GtCO_2e$ in 2030, with increasing traffic demand over the coming decades (UNEP, 2017).

Radiation can be absorbed only if it encounters an appropriate gas molecule in its passage through the atmosphere and into space. The amount of energy absorbed therefore depends on the concentration of those gas molecules: the higher the concentration, the greater the chance of an impact. It follows that as the atmospheric concentration of particular GHGs increases, so does the amount of radiation that gas absorbs (Ahmad and Nour, 2015).

Carbonmonoxide

Carbon monoxide (CO) is one of the most common and widely distributed air pollutants which can affect lifetime of CH_4, CO_2 and also tropospheric (lower atmospheric layer) scavengers. It is a colorless, odorless and tasteless gas that is poorly soluble in water thus plays a role in both air pollution and climate change. Carbon monoxide has a slightly lower density than air and lasts for roughly a month which is long enough for it to be transferred long distances. The annual global emissions of carbon monoxide into the atmosphere have been estimated to be as high as 2600 million tonnes, of which about 60% are from human activities and about 40% from natural processes (Richardson *et al*., 2011).

Anthropogenic emissions of CO originate mainly from incomplete combustion of carbonaceous materials. The largest proportions of these emissions are produced as exhausts of internal combustion engines, especially by motor vehicles with petrol engines, residential heaters like wood-burning stoves. Other common sources include various industrial processes, power plants using coal, and waste incinerators. The other anthropogenic sources are non-road vehicles and engines such as farming and construction equipments, lawnmowers, chainsaws, boats, ships, snowmobiles and aircraft. Some widespread natural non-biological and biological sources, such as plants, oceans and oxidation of hydrocarbons, give rise to the background concentrations outside urban areas. Petroleum derived emissions have greatly increased during the past few decades (IPCC, 2013).

Carbondioxide

Carbondioxide (CO_2) is the largest contributor to global heat warming effect. It plays a pivotal role in the functioning of both natural plant communities and agro-ecosystems. In nature, CO_2 is exchanged continually between the atmosphere, plants and animals through photosynthesis, respiration, and between the atmosphere and ocean through gas exchange (IPCC, 2013). It is considered as a long-lived gas, since a significant fraction remains in the atmosphere 100 – 1000 years after emission (IPCC, 2007b, UNFCCC, 2015).

Human activities have significantly disturbed the nature by extracting long buried fossil fuels and burning them for energy, thus releasing this GHG to the atmosphere. A bulk of extra CO_2 concentration that has been added to the atmosphere is emitted by the burned gasoline of light vehicles, fossil fuel consumption, rapid advancement in industrialization and deforestation which are a cause of global warming (IPCC, 2001, Krupa, 2003).

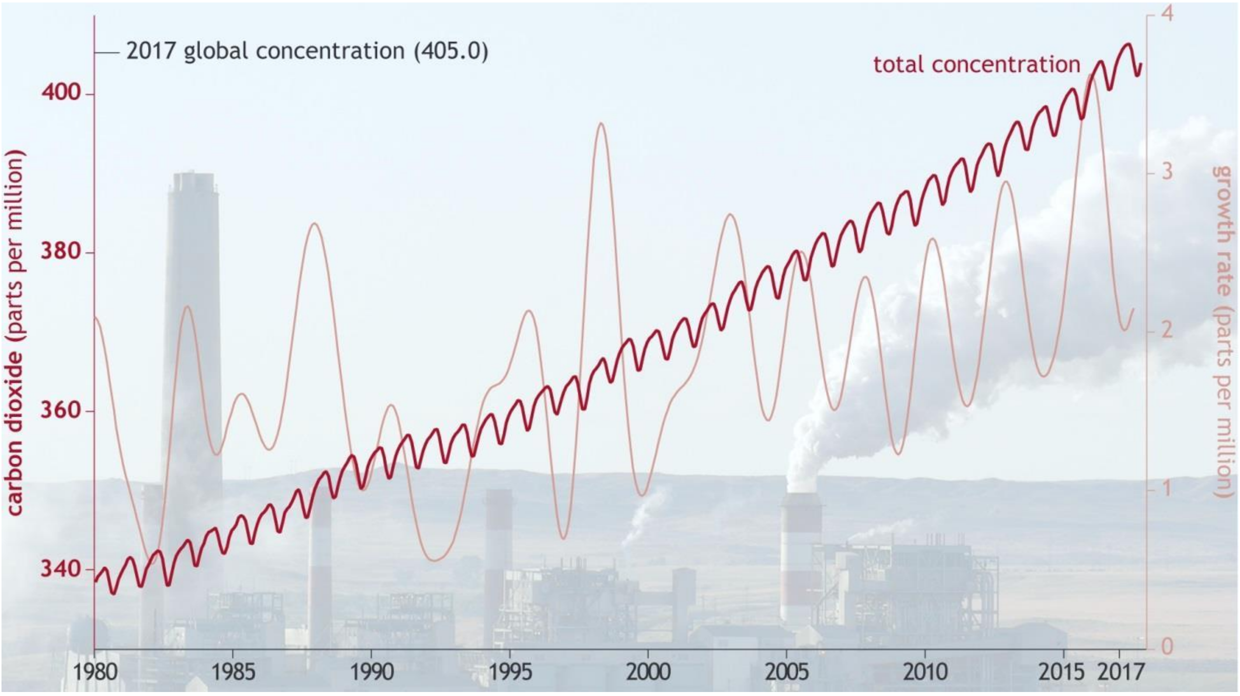

Figure: 2.2. Global atmospheric CO_2 sets a new record high in 2017 (Source: Dlugokencky *et al*., 2018)

From pre-industrial, levels of CO_2 was around 280 ppm and it has increased steadily to 391 ppm in 2009 also the mean temperature has increased by 0.76 ^{0}C over the same time period, 403.3 ppm in 2016 and 405 ppm in 2017 (Olivier *et al*., 2017; Dlugokencky *et al*., 2018). Based on global climate modeling atmospheric CO_2 concentrations may increase over 1000 ppm, and the global surface temperature may also increase by 2.5 ^{0}C to 7.8 ^{0}C by the end of the 21th century (IPCC, 2014).

One of the main constituents of CO_2 is coal, which is very toxic for the environment. Therefore, as noticed, the world's economy depends on fuel that provides energy to the largest number of people in industrialized nations, and then its burning emits the CO_2 that contributes more to the recent increasing in greenhouse warming than any other gas (Ahmad and Nour, 2015).

CO_2 is also released during the burning of agricultural crop waste, such as burning of cereal straw, sugar cane stubble and rice straw. In many countries, it is a common practice to burn large quantities of crop residue, which results killing of insects and other pests as well as disease-causing organisms and neutralizes soil acidity (Cumhur and Malcolm, 2008, Nereu 2005).

According to (Mall *et al*., 2006), the concentration of CO_2 is likely to be doubled by the end of 21th century. High atmospheric temperatures caused by increasing concentrations of CO_2 will induce heat injury and physiological disorders in some crops, which will decrease the incomes of farmers and agricultural countries (IPCC, 2001). Most commonly OTC (Open Top Chamber) and FACE (Free Air Carbondioxide Enrichment) technologies are currently being used for the study of the response of crop plants to the elevated CO_2 (Mall *et al*., 2006).

Methane

Methane (CH_4) is the second largest contributor of changes to GHGs. It is released into the atmosphere by sources like wildfires, soil and natural gas systems, coal mines, landfills, and the raising of livestock (Cumhur and Malcolm, 2008). CH_4 is a short-lived climate gas with atmospheric lifetime of about 10 years. This GHG concentrations contribute about 16% to global warming due to anthropogenic GHG sources and making it the second-leading climate forcer after CO_2 globally (IPCC, 2013).

About one-quarter of the CH_4 emissions caused by anthropogenic activities which comes from domesticated animals, through enteric fermentation, a process whereby plant matter is converted by the methanogenic bacteria. Cattle, goats, sheep, camels, pigs, horses and other animals also emit CH_4. Most of this gas emission from animals is released when manure undergoes anaerobic decomposition by bacteria and other microbes into this gas. The common factors that affect this emission from animals include the feed intake, diet composition, and digestibility of feeds (NMA, 2001).

This gas is also the preventing heat from escaping in the atmosphere; it is the other solid sediments at the ocean which can be classified as natural cause for the global warming. The increase of the ocean floor temperature leads to the melting of this icy solid causing methane bubbles that released in to the atmosphere in order to change the climate (Ahmad and Nour, 2015).

Nitrous Oxide

Concentrations of Nitrous oxide (N_2O) also began to rise at the beginning of the industrial revolution. It is a long-lived climate gas comparing to CH_4 atmospheric lifetime of about 110 years and the most common agricultural based emissions, soil reactions that occur in fertilizer containing N produce N_2O, animal waste and biomass burning (IPCC, 2007b).The main sources of N_2O emissions consist of the manure in pastures, rangeland and paddocks, and synthetic fertilizers (Olivier *et al*., 2017). The chief source of N_2O is microbial activity on organic matter. Ammonia resulting from organic matter or fertilizers after a series of reactions (nitrification and de-nitrification) produces N_2O. Mostly emission level of N_2O depends on the addition of N to soil in any form either organic or chemical N (NMA, 2001).

Halocarbon and Fluorinated Gases

The concentration of halocarbon gases increased primarily due to human activities and natural processes are also a small source. Halocarbons in the atmosphere contribute to ozone depletion and climate change; represent those gases containing chlorine, bromine, fluorine (IPCC, 2007b). Chloro-Fluoro-Carbons atoms that are now controlled substances under the Montreal Protocol due to the discovery that they are able to destroy stratospheric ozone, a global effort to stop their production was undertaken and was extremely successful, so that levels of the major CFCs in the atmosphere are now remaining static or declining (Krupa, 2003).

However, their long atmospheric lifetimes mean that some of the CFCs will remain in the atmosphere for over 100 years. CFCs have no natural source, but were synthesized for use as refrigerants; aerosol and cleaning solvents. The abundance of CFCs is decreasing as a result of international regulations designed to protect the ozone layer (U.S, Environmental Protection Agency, 2006).

Hydrofluorocarbons, perfluorocarbons, and sulfur hexafluoride are also synthetic, powerful GHGs that are emitted from a variety of industrial processes. These gases are typically emitted in smaller quantities, but because they are potent GHG, they are sometimes referred to as High Global Warming Potential gases (IPCC, 2007b).

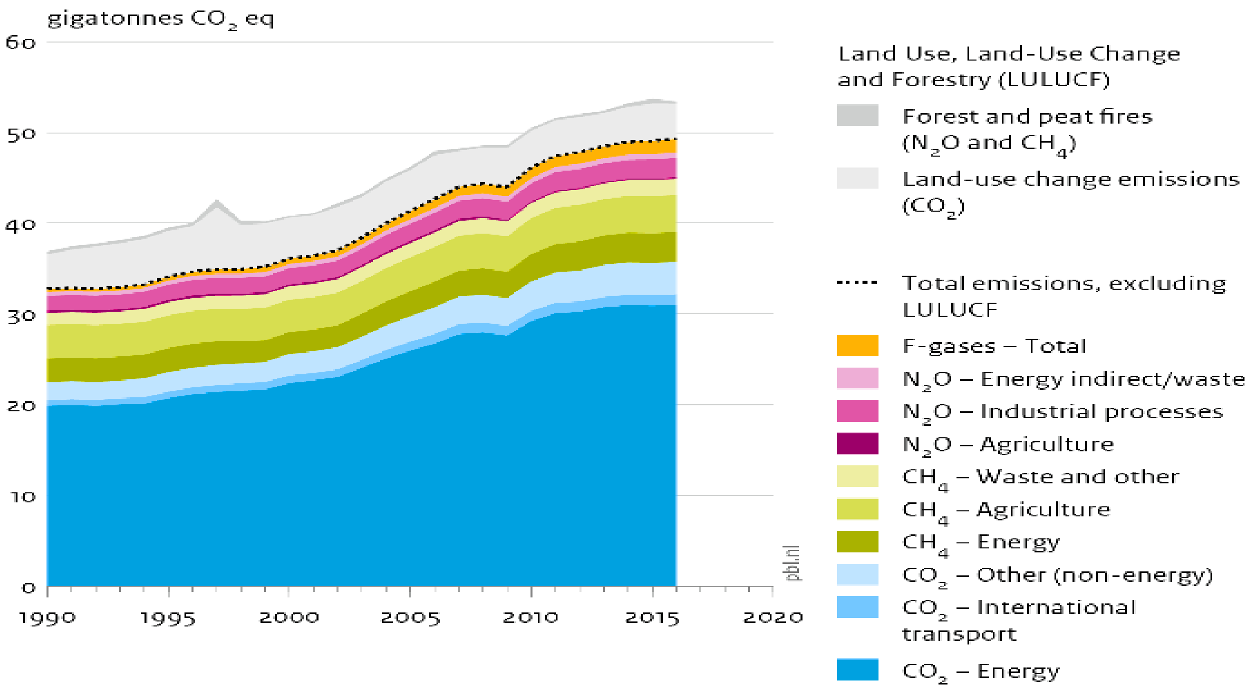

Figure: 2.3. Common global GHGs emissions per type of gas and source (Source: Olivier *et al*., 2017)

2.3. Increasing atmospheric carbondioxide and photosynthesis

Photosynthesis has been recognized as one of the most temperature sensitive physiological processes in plants, that underlie the temperature response of photosynthesis and its acclimation is important to both agriculture and the environment (Wang and Frei, 2011; Vimal *et al*, 2017). CO_2 is a key molecule for photosynthesis and commonly occurs mainly in the leaves. The chemical reaction driven by solar energy involves the reduction of CO_2 through water to create carbohydrates and release oxygen. The resulting carbohydrates are used for plant growth, and provide the energy source for living things (Masahumi *et al*., 2011).

$$\mathbf{CO_2 + H_2O \xrightarrow[\text{Sunlight}]{\text{Chlorophyll}} C_6H_{12}O_6 + H_2O + O_2}$$

Figure: 2.4. Overall chemical equation of photosynthesis (Source: Vimal *et al*., 2017; Taub, 2010)

For photosynthesis to occur, CO_2 must diffuse from the atmosphere towards the chloroplasts. The main gate of entry of this gas into the leaf is via the stomatal pore and an increase in stomatal conductance through the opening of stomata (Taiz and Zeiger, 2006).

The openness of stomata pores through which plants exchange gasses with the external environment is regulating with concentration of this gas. Open stomata allow CO_2 to diffuse into leaves for photosynthesis, but also provide a pathway for water to diffuse out of leaves. Plants therefore regulate the degree of stomatal opening (related to a measure known as stomatal conductance) as a compromise between the goals of maintaining high rates of photosynthesis and low rates of water loss. As CO_2 atmospheric concentrations increase, plants can maintain high photosynthetic rates with relatively low stomatal conductance (Taub, 2010).

At the semi-arid rain-fed site, elevating atmospheric CO_2 concentration increase relative humidity of soil water content of the surface layer (up to a depth of 10 cm), which is beneficial to counteract drought and prolonged the grain filling stage, thereby increasing both grain number and grain weight and, ultimately, grain yield by lower the activity of photosystem in leaves (Gokhale *et al*., 2011).

Increasing atmospheric CO_2 is also enabling a plant to allocate more carbohydrates to its underground parts, thus promoting root growth and the better-developed root systems, in turn, ensures more efficient use of scarce water in times of drought. Thereby promoting drought resistance or drought avoidance due to soil water uptake by leaf area also declined although water is available in adequate amount (Wall, 2001).

Crops sense and respond directly to rising of atmospheric CO_2 through photosynthesis and stomatal conductance, and this is the basis for the CO_2 fertilization effect on crop yield (Long *et al*., 2006). In plants, the promotion of photosynthesis under high atmospheric CO_2 concentration results in increased dry matter production. This is known as the “CO_2 fertilizer effect” and it is used to promote crop growth in greenhouses and plant factories (Amthor, 2001; Prasad, 2005).

Agro-ecosystems may be strongly influenced by the increase in atmospheric CO_2 concentration and associated climate change. The direct effect of increasing CO_2 concentration on plant growth is of particular interest because of the possibility of increasing crop yields in the future once the substrate for photosynthesis and the gradient of this gas concentration between atmosphere and leaf will increase (Nereu, 2005). After long term exposure to high CO_2 concentration the effects on photosynthetic rates will be either positive or negative and will vary among and within species (Gokhale *et al*., 2011).

2.4. Response of three carbon (C_3) and four carbon (C_4) plants to increasing atmospheric carbondioxide

Changes in concentrations of Green House Gases (GHGs) may affect many plant processes, such as photosynthesis, transpiration, nutrient uptake, and senescence, resulting in significant effects on crop growth and yield. Increasing levels of atmospheric CO_2 also causes a decreased stomatal conductance of water vapor. This effect is observed for different species depend on their photosynthetic pathway and CO_2 metabolism (Ainsworth and Mcgrath, 2010).

There are three major categories of CO_2 metabolism: Three carbons (C_3), four carbons (C_4) and Crassulacean acid metabolism (CAM). These are the processes plants use to fix carbon during photosynthesis. Fixing of carbon is the way that plants remove carbon from CO_2 and turn it to carbohydrate. Each responds to elevating of this gas in a different way. CAM photosynthesis is a carbon fixation pathway that evolved in some plants mainly includes succulents. In a plant using full CAM, the stomata in the leaves remain shut during the day to reduce transpiration, but open at night to collect CO_2 (Herrera, 2008).

The pre-collected CO_2 is concentrated around the enzyme Rubisco and it fixed in the cytoplasm of mesophyll cells by a phosphoenolpyruvate (PEP) reaction which is similar to that of C_4 pathway. Unlike the C_4 mechanism, the resulting organic acids are stored in vacuoles for later use that is, they are not immediately passed on to the Calvin cycle (Simpson, 2010; Herrera, 2008). The CO_2 is stored as the four-carbon acid malate in vacuoles at night, and then in the daytime, the malate is transported to chloroplasts where it is converted back to CO_2, which is then used during photosynthesis (Masahumi *et al*., 2011).

Characterizing the response of stomata to elevating CO_2 is important for understanding the response of plants to increasing atmospheric CO_2. Stomata apparently do not respond directly to the CO_2 concentration in the atmosphere around the leaf, CO_2 sensor for stomatal action is considered to be located in the epidermis and is presumably in the guard cells, the inner lateral walls which are permeable to CO_2 (Malin *et al*., 2017). The increase in net photosynthesis in C_3

species has been reported as high as 50% or more when CO_2 concentration doubles than C_4 species (Nereu, 2005).

In C_3 plants, the uptake of this gas by Rubisco is the first step in photosynthetic CO_2 assimilation, and the primary acceptor of CO_2 is ribulose bisphosphate (RuBP) and the enzyme that catalyzes this reaction is Rubisco (Kirschbaum, 2004). As Rubisco catalyzes both carboxylation and oxygenase reactions, both CO_2 and O_2 compete for the same site on Rubisco. Due to competitive interaction, increase in CO_2 will diminish the oxygenase activity of O_2. Therefore, doubling the current CO_2 will increase carboxylation, decrease oxygenation and photorespiration, and thus will increase the rate of net photosynthesis of C_3 species majorly (Prasad *et al*., 2005).

Around 85% of plants on earth use C3 path to fix carbon via Calvin cycle and produce 3 carbon molecules known as 3-phospo-glyceric acid. Common C_3 crops are small grain cereals (wheat, rice, barley, oat and rye), legumes or pulses (soybean, peanut, various beans and peas, nut); root and tuber crops (potato, cassava, sweet potato, sugar beet, yams) (Kajala *et al*., 2011).

Plants with C_4 photosynthesis will respond little to rising atmospheric CO_2 compared to C_3 plants because of a mechanism to increase the concentration of CO_2 in leaves causes CO_2 saturation of photosynthesis of these plants. Some examples of C_4 crops are maize (corn), sugarcane, sorghum, millet, and many tropical and subtropical zone (warm-climate) grass species (Long *et al*., 2004; Leon and Vara, 2014).

Atmospheric CO_2 concentration about (360 µl/liter) is insufficient to saturate the Rubisco, the enzyme responsible for primary carboxilation; the metabolic process that drives photosynthesis in C_3 plants (Nereu, 2005). There is growing evidence suggesting that many crops, notably C_3 crops may respond positively to increase atmospheric CO_2 in the absence of other stressful conditions (Long *et al*., 2004).

However, if global warming will take place, an increase in temperature may offset the benefits of increasing CO_2 on crop yield and current atmospheric CO_2 is sub-optimal for photosynthesis of C_3 plant species (Kirschbaum, 2004; Nereu, 2005).

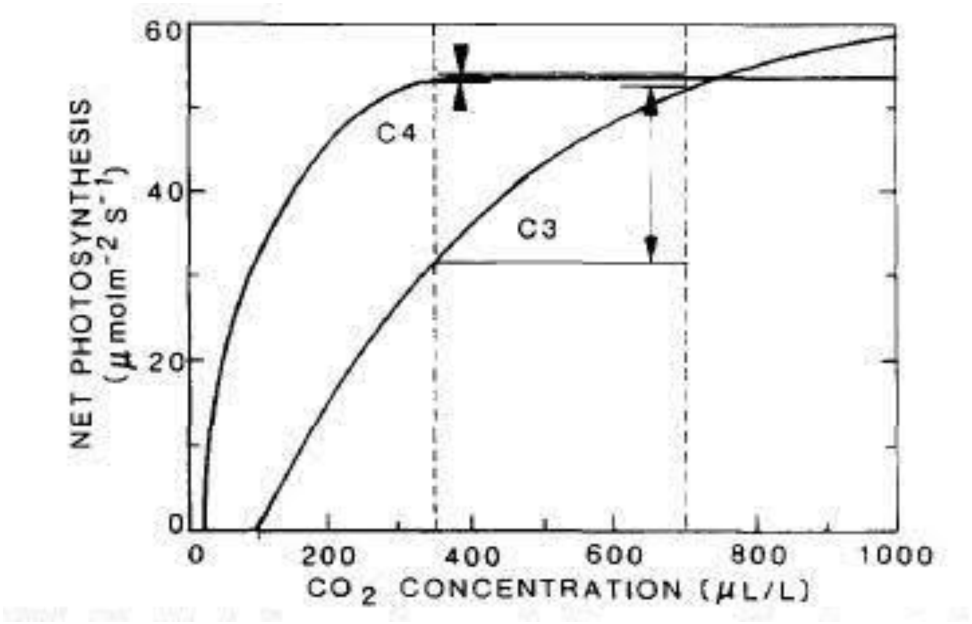

Figure: 2.5. Net photosynthesis curves for C_3 and C_4 species. Arrows indicate increment rise in net photosynthesis due to atmospheric CO_2 doubling (Source: Masahumi *et al*., 2001; Kimball *et al*., 2003).

The efficiency of the C_3 pathway decreases as atmospheric CO_2 decreases (James *et al*., 2002).In C_4 species photosynthesis is likely to be CO_2 saturated at low concentrations due to the mechanism for concentrating CO_2 around rubisco and crops under this photosynthetic path way would not benefit much from increment of this GHG in atmosphere (Ainsworth and Rogers, 2007).

C_3 photosynthesis only uses the Calvin cycle for fixing CO_2 catalyzed by Rubisco, which takes place inside of the chloroplast in mesophyll cell. For C_4 plants such as maize photosynthetic activities are partitioned between mesophyll and bundle sheath cells that are anatomically and biochemically distinct (Wang *et al*., 2012; Kajala *et al*., 2011).

The initial carbon fixation is catalyzed by phosphoenol-pyruvatecarboxylase (PEPC) forming oxaloacetate from CO_2 and PEP. Oxaloacetate acid is metabolized into malate, and then diffuses into the bundle sheath cell where it is decarboxylated to provide increased concentration of CO_2around rubisco. Finally, the initial substrate of the C_4cycle phosphoenolpyruvate is regenerated in mesophyll cell by pyruvate orthophosphate dikinase (PPDK) (Prasad *et al*., 2005).

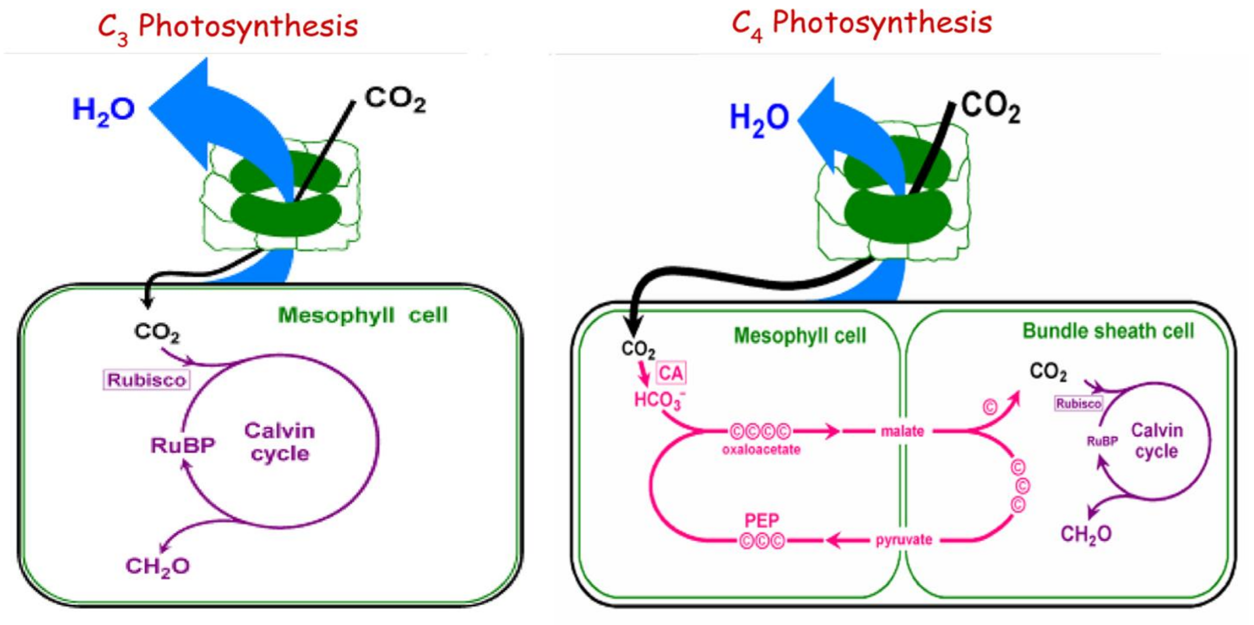

Figure: 2.6. A schematic diagram of C_3 and C_4 photosynthesis (Source: Wang *et al*., (2012))

Crops such as wheat, rice, barley, oat, and rye, grain legumes or pulses soybean, peanut exposed to elevated CO_2 generally grow larger and have a higher percentage of total biomass in stems to support leaves and seed pods. Crops such as rice and wheat produce a larger number of tillers, which leads to greater yield because of the greater number of seed heads per plant. Leaves may be larger or thicker and accumulate more starch (Leon and Vara, 2014).

The biochemical basis for the elevating atmospheric CO_2 stimulation of C_3 photosynthesis is that high CO_2 around rubisco accelerates the carboxylation reaction, reducing the CO_2 loss and energy costs associated with photorespiration, therefore accumulation of carbohydrates in leaves is one of the most pronounced and universally observed responses of C_3 plants to elevated atmospheric CO_2, in field-grown plants where rooting volume is unrestricted (Long *et al*., 2004; James *et al*., 2002).

Due to increment in atmospheric CO_2 increases in seed yields of many C_3 crops range between 20% and 35%, whereas increases for C_4 crops are about 10% to 15% (Ainsworth *et al*., 2002). The effect of CO_2 fertilization remains uncertain but important depending on plant photosynthesis type; two of the most crucial metabolic functions in determining the magnitude of plant growth are carbon assimilation and water use efficiency (Kirschbaum, 2004; Wang and Frei, 2011; Masahumi *et al*., 2011).

2.5. Impact of increasing atmospheric carbondioxide on nutritional composition of edible grains

Climate change impacts on agriculture are expected to undermine human health by affecting the affordability and availability of nutritious food. While levels of under-nutrition are already high across the sub-Saharan African region and projections indicate that with warming of 1.2–1.7 ^{0}C by 2050, the proportion of the population that is undernourished would increase by 25–90% compared to the present (Lloyd *et al*., 2011).

Nitrogen (N) availability to plants determines their responses to increasing atmospheric CO_2 more than any other environmental factor and this has been associated with the fact that increasing atmospheric CO_2 results in decreased N content, this is because that much amount of N is able to be diluted due to, the accumulation of carbohydrates (sugars and starches) per unit leaf area increase on average by 30–45% (Leakey *et al*., 2009).

Reduced uptake of N from the soil under high CO_2 due to lower transpiration rates resulting from decreased stomatal conductance and by impaired nitrate assimilation associated with decreases in the photorespiration pathway at elevated CO_2, as found in C_3 species (Fabio *et al*., 2010). Other than dilution of N from increased carbohydrate concentrations decreased uptake of minerals from the soil is also the other impact of increasing atmospheric CO_2 which is due to increasingly scarce resource or by effects on the shoot system mineral assimilation capacity due to physiological change followed the elevating atmospheric CO_2 (Taub, 2010).

The effects of elevated CO_2 on protein concentrations are likely to be larger for non-leguminous crops and smaller for leguminous crop due to physiologically consistent with the general ability of leguminous crops to match the stimulation of photosynthetic carbon gain at elevated CO_2 with greater nitrogen fixation, to maintain tissue C:N ratios (Rogers *et al*,. 2009). The mechanism(s) by which increasing atmospheric CO_2 decreases tissue concentrations of N and protein are not thoroughly understood, as growth at elevated CO_2 can affect multiple processes involved in nitrogen uptake and metabolism (Gifford *et al*., 2000).

Decreased protein content in non-leguminous C_3 crops may have serious consequences for public health especially for the future (Swaminathan *et al*., 2012). Declining in nutrient concentrations and composition associated with elevating atmospheric CO_2 is due to carbohydrate dilution by which CO_2 stimulated carbohydrate production by plants dilutes the rest of the grain components (Fabio *et al*., 2010).

Decreases in leaf protein can lead to decrease seed protein concentration as the N supply to seeds during filling is largely from translocation of catabolized proteins in senescing photosynthetic tissues (Salon *et al*., 2001). Dilution of protein by elevating CO_2 results if the increase in biomass accumulation is larger than the increase in N acquisition. This effect is referred to as growth dilution and leads to reduced concentration of protein with increased yield (a common effect of elevated atmospheric CO_2) (Ainsworth and Long, 2005).

Failure of Nitrogen acquisition to keep pace with growth enhancement can be caused by N becoming a scarce resource or by effects on the shoot system nitrate assimilation capacity and by the root system nutrient uptake capacity. Grain grown in the elevated CO_2 atmosphere produced poorer dough, decreased loaf volume, farinograph development time, and dough extensibility due to reduction of protein in there grains flour (Johan and Kanplei, 2012).

Food crops such as wheat, rice, barley, soya, maize and field peas, which serve as an important source of dietary zinc and iron for billions of people around the world, have recently been shown to contain lower concentrations of zinc and other micronutrients when grown under open field conditions at a higher concentration of CO_2 atmosphere (Stoltzfus *et al*., 2004). Dietary deficiencies of zinc and iron are currently responsible for large burdens of disease globally and the populations who are at highest risk of these deficiencies also receive most of their dietary zinc and iron from crops (Samuel *et al*., 2015).

Increasing CO_2 content of the atmosphere affect populations consuming different plant foods, it was found that Zinc and Iron content of C_3 plants and pulses can decrease significantly, which would affect billions of people, by increasing malnutrition in terms of these two elements (Zoltan, 2018). An estimated two billion people suffer these deficiencies, causing a loss of 63 million life years annually and most of these people depend on C_3, C_4 cereals and legumes as their primary dietary source of micronutrients (Stoltzfus *et al*., 2004; WHO, 2007).

The effects of atmospheric CO_2 concentration on crop yield will become greater as the atmospheric CO_2 concentration increases. For many crops, the predicted yield increase in response to a 700 ppm CO_2 concentration is approximately 30%; specifically, a yield increase of

31% in wheat, 29–35% in rice, 55% in soybean and 50% in barely. However, the promoting effects will vary according to the specific nature of the plant and on the other side negative change in nutrients also increase with the yield (Masahumi *et al*., 2011).

Fruits like strawberry with a C_3 photosynthetic pathway respond favourably to increases in atmospheric CO_2 concentrations. As a result, CO_2 enrichment of horticultural crops, including strawberry, grown in protected production systems is gaining interest, mostly in temperate regions during winter through spring to increase yields (Amatori *et al*., 2016). According to Balasooriya *et al*., (2017), strawberry fruits grown under higher CO_2 levels contained significantly greater levels of flavor compounds compared to the fruits under normal (Ambient) levels of this gas. The concentrations of ethyl hexanoate, ethyl butanoate, and methyl hexanoate increased by 48%, 35%, and 68%, respectively, at the highest CO_2 level (950 ppm). Moreover, CO_2 at 950 ppm lowered organic acid (citric and malic) contents by 17% and increased total sugar to organic acids ratio by 40%, which represents a reduction in fruit sourness.

2.6. Emission of Greenhouse Gases in Ethiopia

(European Union, 2011) reported that Ethiopia faces uncertainty over rainfall and climate models suggest that the country will see further warming of between 0.7 and 2.3 ^{0}C by 2020. Climate change has already led to an increasing number of hot days and the effects on crop. The majority of the Ethiopian population, over 85% depends on rain fed agriculture (MoFED, 2009).The proliferation of GHGs is mainly due to increased anthropogenic activities, including use of fossil fuels in transport and energy generation, land use changes and deforestation due to expansion of agriculture and settlements.

According to (Ethiopia’s climate resilient green economy, 2011) emission intensive sectors that account for a large share of total emissions in high-emitting countries, such as industry and power, currently make up a much smaller share of Ethiopia’s national emissions. Industries contribute approximately 1% of the national emissions, most of which comes from the construction sub-sector. The power sector also accounts for a small share of energy sector emissions as Ethiopia’s grid is largely reliant on hydropower. The energy sector overall emits close to 15% of the national emissions and a major share of this is from transportation. Close to 80% of the national emissions originate in the agriculture like crop production, livestock, and manure. Similar to the rest of African countries, it’s expected to change as socio-economic development progresses. Ethiopia is experiencing high rates of urban population growth of up to 4.4 % per year (NMA, 2001; Second National Communication of Ethiopia to UNFCCC, 2015).

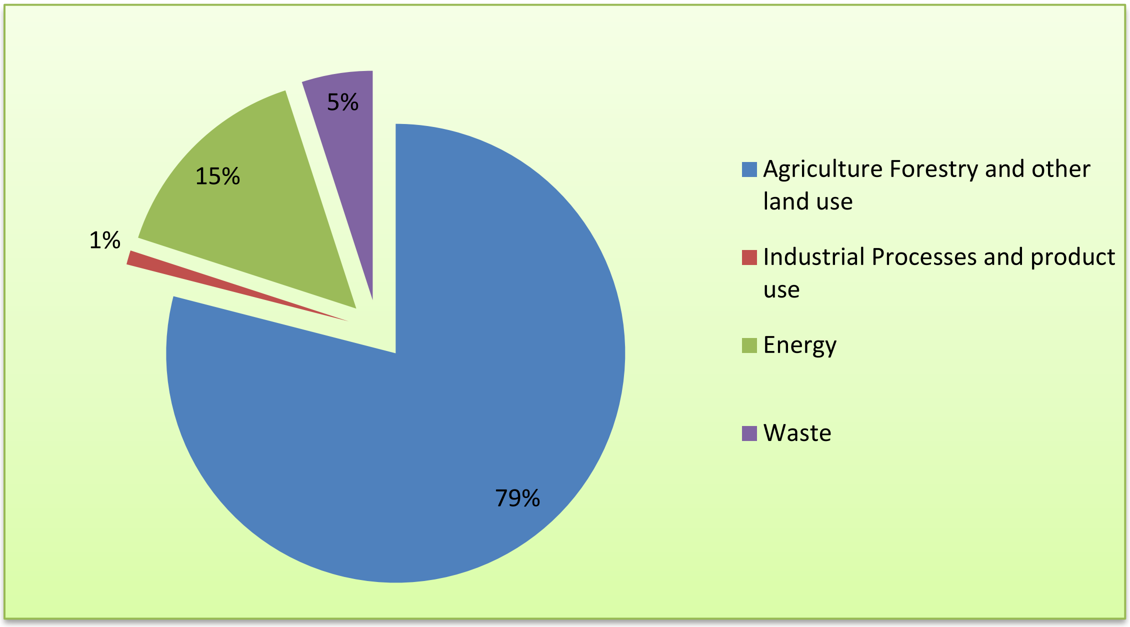

Figure: 2.7. Overview of Ethiopia's emissions profile and major contributing sectors (Source: Second National Communication of Ethiopia to UNFCCC, 2015)

Due to concerns arising from the risk of global climate change, the UN General Assembly established the Intergovernmental Negotiating Committee for a Framework Convention on Climate Change (INCFCCC) in 1990. These efforts succeeded, and the UNFCCC came into force on 21 March 1994. After the first National Communication since 1994 the Second National Communication (SNC) has been compiled to meet Ethiopia's obligations under the UNFCCC. It's describes national progress made to implement the Convention since 1994 to 2013 G.C.

According to (UNFCCC, 2015), in 2013, Ethiopia's national emissions stood at 150 Mt CO_2 emissions which is 0.3 % of global emissions 34.5 (Bt) Billion tons of CO_2. If current practices prevail, GHGs emissions in Ethiopia will more than double to 400 (Mt) Metric tons of CO_2 in 2030.

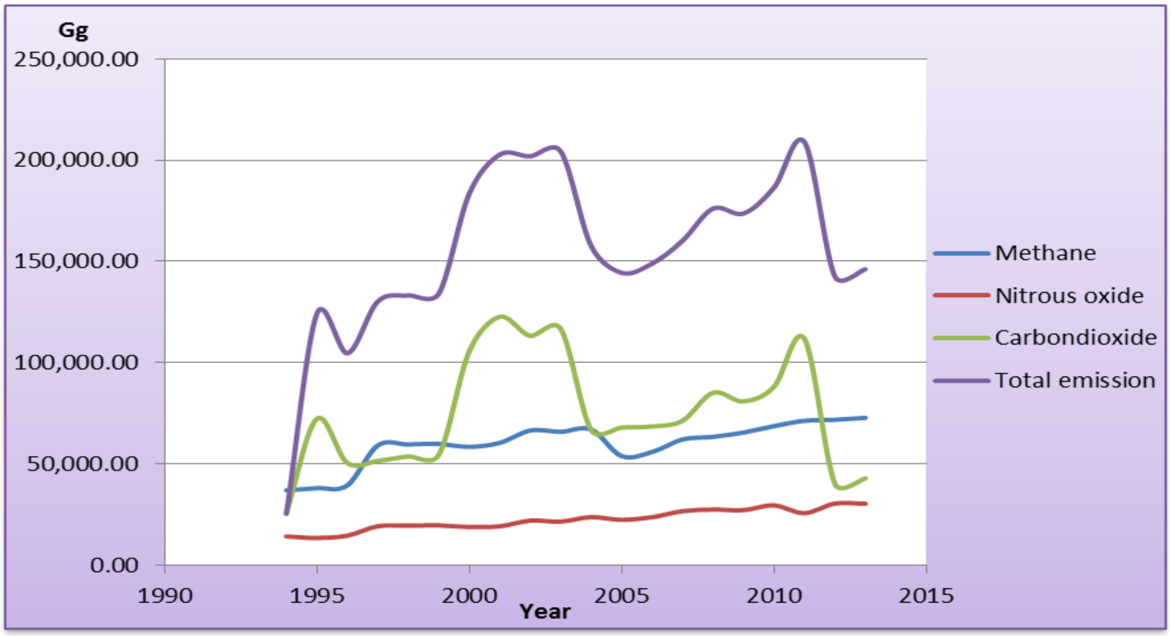

Figure: 2.8. Green House Gases emissions in Ethiopia (Gigagram), from 1994 to 2013 G.C (source: Initial National Communication of Ethiopia, 2001; Second National Communication of Ethiopia to UNFCCC, 2015)

Developmental pressures and the economic vision to achieve middle income status by 2025 are expected to increase demands for energy intensive infrastructure in the country and as per official estimates, the industrial sector, followed by transport, are expected to register the largest annual growth in emissions (Ethiopia's climate resilient green economy, 2011).

2.7. Temperature and rain fall pattern of Ambo, West Shoa Zone of Oromia region

Fluctuations of temperature and rainfall pattern are the common climate change indicators. Ethiopia's contribution to GHG emissions is very low on a global scale. The emissions of GHGs are predominantly from high income countries while the negative effects of climate change are strongly in low income countries like Ethiopia. This means climate change is generally expected to hit developing countries harder than industrialized countries this is because that they are less capable of mitigating or adapting to the changes due to their poverty and high dependence on the environment for subsistence.

Similarly, the main part of the Ethiopian economy is rain-fed agriculture, which is heavily sensitive to climate variability and change. Clearly Ethiopia is highly vulnerable to current variability and there are also indications that climate change will increase rainfall variability which will likely increase losses from rain-fed agriculture (NMA, 2007). The following two tables show the monthly rainfall pattern and temperature records in the past three decades report from Ambo agriculture station of Ethiopian national metrological agency.

Rain-fall pattern (mm)												
Year (G.C)	**Months**											
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
1987	1.6	38.1	128.3	119.8	185.4	275.2	315.9	196.1	151.6	13.6	1.4	12.7
1992	11.5	58.7	152.4	181.9	121.2	229.1	263.5	169.0	121.3	25.1	0.0	0.0
1997	28.7	0.0	71.0	83.1	59.7	132.0	132.1	183.4	59.2	69.0	22.1	7.2
2002	58.7	16.9	55.6	56.3	39.5	178.0	164.2	142.7	40.3	17.9	0.0	17.2
2007	19.3	54.3	83.2	38.9	131.6	178.3	152.6	218.3	111.7	11.0	0.0	10.0
2012	0.3	0.0	3.6	56.5	23.1	142.5	283.1	367.7	173.1	53.2	0.0	18.0
2017	0.0	0.0	11.4	27.5	118.9	89.0	152.3	140.9	129.6	7.6	17.6	25.0

Table: 2.1. Monthly report of rainfall pattern records from Ambo Agriculture station in the past thirty years (Source: NMA, 2017).

The National Metrological Agency (2001) revealed that in Ethiopia climate variability and change is mainly manifested through the variability and decreasing trend in rainfall and increasing trend in temperature. Historical climate analysis for Ethiopia indicates that annual temperature has increased by 1.3°C between 1960 and 2006, an average rate of 0.28°C per decade. The increase in temperature in Ethiopia has been most rapid in June, August, and September at a rate of 0.32°C per decade. The rainfall is historically highly variable and there is no clear trend in the amount of rainfall over time (NAPA, 2007).

Temperature (^{0}C)													
Year (G.C)		**Months**											
		Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
1987	max	25.9	26.4	26.6	26.7	26.9	24.3	22.6	22.4	23.8	25.6	24.4	25
	min	11.4	12.9	13.2	13.4	13.5	11.7	8.9	10.8	7.9	10.3	11.4	11.2
1992	max	26.2	26.3	26.8	27	26.8	23.6	22.4	22.1	23.3	22.7	25.5	26.3
	min	10.9	10.6	12.7	12.5	12.7	11.6	11.8	11.3	11.2	10.9	11.9	11.4
1997	max	26.9	27.5	27.3	28.1	26.9	24	23.3	24.3	23.8	24.2	26.7	27.8
	min	10.3	11.9	12.9	13.1	12.3	12	12	11.1	10.1	10.6	11	10.3
2002	max	27.1	27.6	28.7	28.4	29	25.2	23.9	24	24.3	26.5	27.6	28.1
	min	11.5	12.5	11.9	12.7	13	12.1	12.1	11.2	11.5	10.7	11.6	11.5
2007	max	27.4	28.2	28.9	28.9	28.9	24.9	22.4	24.3	24	25.7	27.9	27.8
	min	12.6	11.9	13	13.5	13.5	12	12.6	12.5	12.3	10.1	11.4	11.3
2012	max	28.4	28.9	29.1	29.7	29.5	25.7	24.7	25	27.5	27	28.2	29.7
	min	12.2	12.9	14.1	13.9	13.8	12.2	12.9	12.7	12.1	10.6	11.3	11.8
2017	max	30.6	29.7	29.9	32	30.1	27.1	29.2	28.3	28.9	27.3	28.9	31.7
	min	9.6	13.8	14	15.2	13.9	12.6	14.1	13.8	13.6	12.7	14	12.6

Table: 2.2. Monthly report of temperature from Ambo Agriculture station (Source: NMA, 2017)

CHAPTER THREE

3. Materials and Methods

3.1. Study Area

The study was conducted in Addis Ababa the capital city of Ethiopia. The samples were collected from Ambo, West Shoa Zone, Oromia region and the experiments were carried out from November 2017 to June 2018. The proximate composition except fiber was carried in Addis Ababa University Center for Food Science and Nutrition, Laboratory. Mineral contents (Calcium, Zinc, Iron and Magnesium) and crude fiber analysis was done in Jije Labo glass enterprise and Ethiopian public health institute (EPHI) laboratories, respectively.

3.2. Method of Sample and Sampling

Ethiopian biodiversity institute (EBI) has seven additional biodiversity centers other than Addis Ababa, in Metu, Hawassa, Harer, Mekele, Goba, Bahirdar, Asossa and botanical gardens in Jimma, Shashemene and Fiche duplicate gene bank.

The samples for this study were taken from Addis Ababa branch of the EBI gene bank. The two samples were taken from the selected bag based on the years, accessions and region (Ambo District, West Shoa Zone of Oromia region, Amaro kebele). The samples were harvested with five years interval, totally 200 gm of *Triticum aestivum* L. and 250 gm of *Zea mays* L. from each year was taken. The samples was packed in polyethylene bags and transported to Addis Ababa university Center for Food Science and Nutrition laboratory by a vehicle. Samples were well cleaned before they stored in gene bank of EBI and suitable for any type of laboratorial analysis therefore, they directly goes to be milled by laboratory mill (FW100, China) and flour were packed in tight polyethylene bags and store in cool dry place (shelf) until the analysis.

Germination capacity of very samples of *Triticum aestivum* L. and *Zea mays* L. from 1987-2017 G.C was already determined and filed by the institute itself during the collection of each gene from different areas of the country and all of the collected sample from EBI was have range from 80 to 90.5% and 89.8 to 92% capacity of germination for *Triticum aestivum* L. and *Zea mays* L. respectively (EBI, 2017).

Accession	**Year (G.C)**	**Region**	**Zone**	**Locality**	**Kebele**	**Species name**	**Germination capacity (%)**
203883	1987	Oromia	Mirab-Shoa	Ambo	Amaro	*Triticum aestivum*	90.5
226898	1992	Oromia	Mirab-Shoa	Ambo	Amaro	*Triticum aestivum*	89.7
228755	1997	Oromia	Mirab-Shoa	Ambo	Amaro	*Triticum aestivum*	86.5
228778	2002	Oromia	Mirab-Shoa	Ambo	Amaro	*Triticum aestivum*	84.6
228801	2007	Oromia	Mirab-Shoa	Ambo	Amaro	*Triticum aestivum*	82.1
228964	2012	Oromia	Mirab-Shoa	Ambo	Amaro	*Triticum aestivum*	80.23
231521	2017	Oromia	Mirab-Shoa	Ambo	Amaro	*Triticum aestivum*	80
237602	1987	Oromia	Mirab-Shoa	Ambo	Amaro	*Zea mays*	92
237694	1992	Oromia	Mirab-Shoa	Ambo	Amaro	*Zea mays*	91
239647	1997	Oromia	Mirab-Shoa	Ambo	Amaro	*Zea mays*	92.1
239680	2002	Oromia	Mirab-Shoa	Ambo	Amaro	*Zea mays*	90.7
239697	2007	Oromia	Mirab-Shoa	Ambo	Amaro	*Zea mays*	90.1
239708	2012	Oromia	Mirab-Shoa	Ambo	Amaro	*Zea mays*	89.8
239756	2017	Oromia	Mirab-Shoa	Ambo	Amaro	*Zea mays*	90

Table: 3.1. Germination capacity of collected samples **(**Source: Ethiopian Biodiversity Institute, 2017)

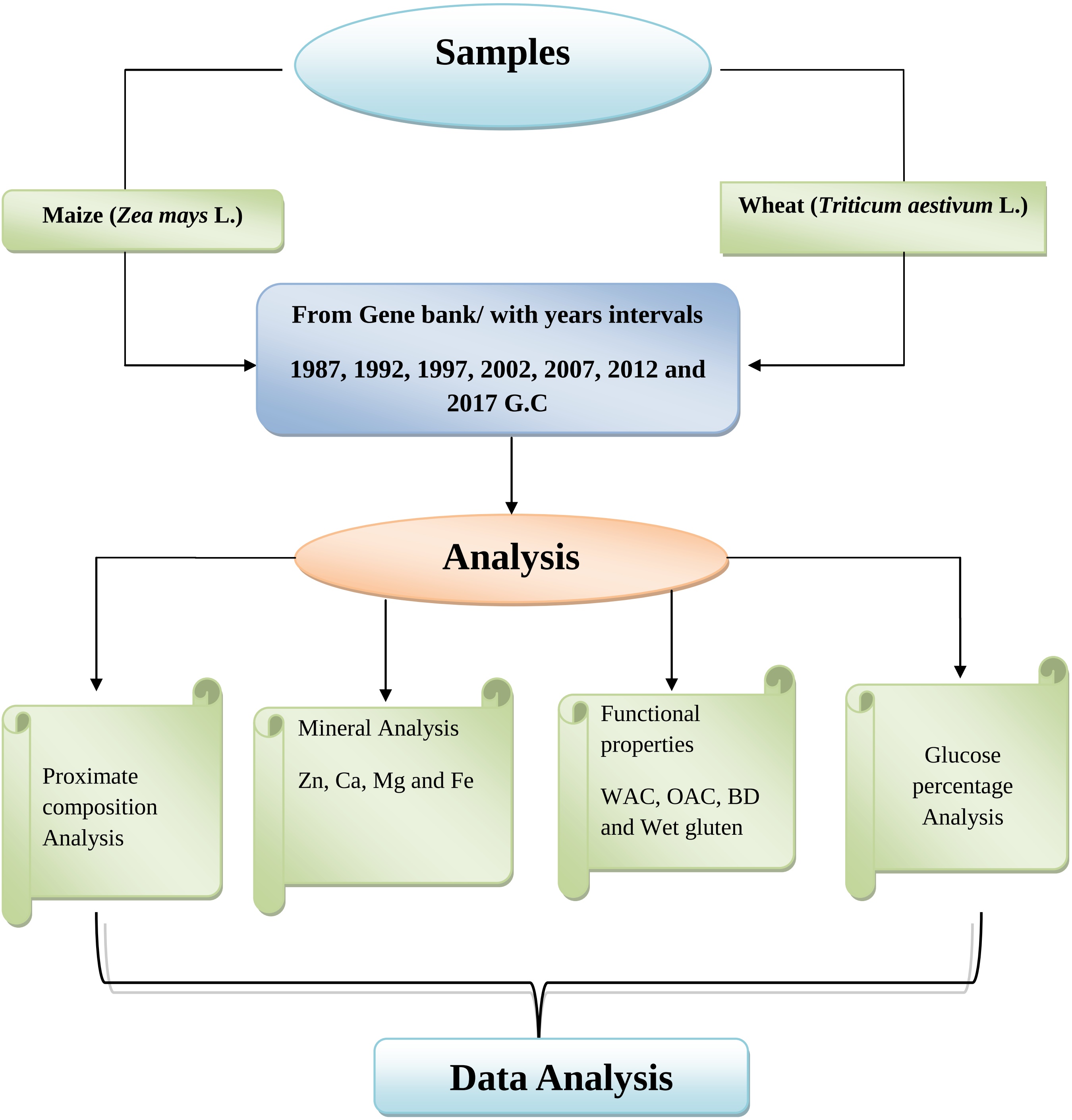

Figure: 3.1.Experimental framework of this study

3.3. Proximate composition analysis

Moisture Content

Moisture content was determined by AOAC 2010 Method number 925.10. The aluminum dishes use for the moisture determination was dried at 100 ^{0}C for 1hr by using oven (DHG – 9123A, Sweden). The dishes were re moved and kept in a desiccator for about 30 minutes. The mass of empty dishes was measured as M_1. About three gram of the powder sample was weighed using analytical balance (Model ARA520, USA) in to the dish and recorded as M_2 and dried at 105 ^{0}C for 3 hrs. And it was cooled in a desiccator then it was weighted again as M_3. This process was repeated until constant weight obtained. Then, moisture content was calculated by using the following formula:-

$$\text{Moisture (\%)} = \frac{M2-M3}{M2-M1} * 100 \qquad \text{(Equation 3.1)}$$

Where: M_1 = mass of empty dried dishes, M_2 = mass of dish and sample before drying and M_3 = mass of dish and sample after drying

Crude Fat

Crude fat content was determined by AOAC 2000 method number 4.5.01. The flasks use for the extraction was washed and then dried in oven (DHG – 9123A, Sweden) at 70 ^{0}C for 1hr and cool in a desiccator. The masses of the cooled flasks were measured by analytical balance (Model ARA520, USA) recorded as (M_1). About two gram of the powder sample was weighed (M) in to each thimble lined with cotton at their bottom. The thimbles with its sample content were placed in to the Soxhlet extraction apparatus (FOSS Soxtec^{TM} 8000 Extraction unit, Sweden). Then 50 mL of petroleum diethyl ether was added in to each flask and the extraction process was carried out for about 3 hrs with boiling, rinsing and recovery processes. Then the flasks with their content were removed from the Soxhlet and placed in drying oven at 105^{0}C for 30 minutes. The flasks with their contents were then placed in a desiccator for 30 minutes. The mass of each cooled flask together with its fat contents were measured as (M_2). Then, the total lipid amount will be calculated by the following formula:-

$$\text{Crude fat content (\%)} = \frac{M2-M1}{M} * 100 \qquad \text{(Equation 3.2)}$$

Where: M_2 = mass of flask and lipid extracted, M1= mass of dried flask and M = weight of sample on dry basis

Crude Protein

Crude Protein was determined by AOAC 2000 method number 979.09. About one gram of powder sample was weigh on analytical balance (Model ARA520, USA) and put it in Tecator tube then, transfer to the tecator rack. The Kjeldahl protein analyzer instrument (FOSS Kjeltec - 8460 Analyzer unit, Sweden) was used. Then 6 mL of acid mixture (5:1 Conc. H_3PO_4: H_2SO_4) and 3.5 mL of 30% H_2O_2 mixed immediately sample and acid carefully and 3.55 mL of 30% H_2O_2 was added in to the digestion flask step by step. The tubes were shaked and watched out for a violent reaction. After this violent reaction was disappeared and added three gm of the catalyst mixture (0.5:100 Se: K_2SO_4) was added in to the digestion flask and stand for 5-15 minutes before digestion. The solution was then digests at 370 ^{0}C for 1 hrs and cooled. After digestion is completed the distillation was takes place 50 mL of distilled water was then added, the solution was then shake to avoid precipitation of sulphate in the solution, and then 25 mL of 40% sodium hydroxide was added to neutralize the acid and to make the solution slightly alkaline. A 250 mL of conical flask was placed and contacted 25 mL of boric acid, 25 mL of distilled water and indicator solution under the condenser of the distiller with its tip immersed in to solution. The distillation was continued until a total volume become between 200 and 250 mL, borate ion was formed as a result of the reaction of the boric acid and the ammonia then; rinse the tip with distilled water before the receiver is removed. And this was titrated with standard acid (0.1N H_2SO_4) until the green color changed to reddish color. The total N content was calculated by using the following formula:-

$$\text{Nitrogen (\%)} = \frac{(V2 - V1) * N * 14 * 100}{M} \quad \textbf{(}\text{Equation 3.3)}$$

Where: V_1 = Volume in ml of the standard Hcl used in the titration for the blank, V_2 =Volume in ml of the standard Hcl used in the titration for the test material, N = Normality of the standard hydrochloric acid, 14= Molecular weight of nitrogen and M= Weight of sample on dry basis

Protein (%, w/w) = %N * conversion factor

Total Ash

Total ash content was determined by AOAC 2000 method number 923.03. A porcelain crucible used for the analysis was cleaned and dried in a muffle furnace (Model, DHG – 9123A) for 30 minutes at 550 ^{0}C. The crucible was cooled in a desiccator for about 30 minutes and weighed (M_1). Two gram of powder sample was added in to each crucible and weighed (M_2) by analytical balance (Model ARA520, USA). The crucible with powder sample was then placed on a hot plate at 120 ^{0}C for 4 hrs in a hot plate under a fume hood until smoking ceases and the samples become thoroughly char. Then the sample was ashed in a muffle furnace at 550 ^{0}C for 5 hrs until the ash became clean and white in appearance. Finally, the crucible with ash was removed from the muffle and place in a desiccator for 1 hr to cool and after cooled it was re-weighed (M_3). Then, the total ash content was obtained by the following formula:-

$$\text{Total ash (\%)} = \frac{(M3-M1)}{M2-M1} * 100 \qquad \text{(Equation 3.4)}$$

Where: M1= mass of the dried dish, M2 = mass of the dish and the sample on dry basis and M3=mass of the dish and the ash

Crude Fiber

Crude fiber was determined AOAC 2016 method number 962.09. By using analytical balance (Model ARA520, USA), two gram of the sample was weighed in each of crucible (M_1) and inserted with 600 mL beaker. Fiber analyzer instrument used was (Fibertech^{TM} 2010, USA). A 200 mL of 1.25% of sulfuric acid solution was added to each beaker and allowed to boil in crude fiber apparatus for 30 minutes by rotating and stirring it periodically during boiling the level was kept constant by addition of hot distilled water. After 30 minutes, 20 ml of 28% potassium hydroxide was added in to each beaker and again allowed to boil for another 30 minutes. The level was still kept constant by addition of hot distilled water. The solution found in each crucible was filtered via funnel fitted with rubber stopper. During filtration the sample residue was washed with hot distilled water; with 1% sulfuric acid solution, hot distilled water, 1% NaOH and finally with acetone. Each of the crucibles with their content was dried at 130^{0}C for 2hrs in dried oven and cooled in a desiccator and weighed as (M_2). Then again they were ashed for 30minutes at 550^{0}C in muffle furnace and were cooled in a desiccator. Finally the mass of each crucible was weighed as (M_3). The crude fiber content was calculated by using the following formula:-

Crude fiber content (%) = $\frac{(M2- M3)}{M1}$*100 (Equation 3.5)

Where; M_1 = weight of sample on dry basis, M_2 = weight of the crucible and sample after drying and M_3= mass of the crucible and sample after ashing

Utilizable Carbohydrate

The Utilizable carbohydrate was calculated by difference. The mathematical expression was as follows:-

Utilizable Carbohydrate (%) =

100 – (Crude Fat + Crude Protein + Crude fiber + Total Ash) (Equation 3.6)

Total Energy

The total energy content in each sample was calculated as follows:

Energy (kcal/100g) = (9* % Fat + 4* % Protein + 4* % Carbohydrate) (Equation 3.7)

3.4. Phenol Sulphuric Acid Method for Determination of Total Glucose

Principle

In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This forms a green coloured product with phenol and has absorption maximum at 490 nm.

Materials

Phenol 1%: Redistilled (reagent grade) phenol 1mL and make up to 200 mL by distilled water

Sulphuric acid: 96% reagent grade.

Standard glucose: Stock twenty five milligram in 100 mL of water. Working standard 10 mL of stock diluted to 100 mL with distilled water.

Phenol sulphuric acid method for determination of total glucose was done by Elena, (2012). Sixty milligram of the flour samples of *Triticum aestivum* L. and *Zea mays* L. was weighed by electronic balance (Model AX120, China) into a boiling tube which was well cleaned and dried. Then hydrolysed by keeping it in a boiling shaking water bath (Model, YCM – 012S, USA) for 3 hrs with 5 mL of 2.5 NHCl and cooled to room temperature. The hydrolysed sample was neutralized with solid sodium carbonate until the effervescence ceases. Make up the volume to 100 mL and centrifuged (Model, 300D, China) at 3500 rpm for 20 minutes and pipetted out 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard into a series of test tubes. The neutralized sample was pipetted out 0.2 mL and make up the volume of tube to 1 mL with distilled water. The blank was prepared with 1 mL of water and 1 mL of phenol solution was added to each tube followed by 5 ml of 96% sulphuric acid to each tube including the blank and shaked well. Then after 10 minutes shake the contents in the all tubes, placed in a shaking water bath (Model, YCM – 012S, USA) at 25–30 ^{0}C for 20 minutes and finally read the colour at 490 nm by using UV/Vis/NIR Spectrometer – STD detector module (Model – Lambda 950, England). Amount of total glucose presented in the sample solution was calculated by using the standard graph.

Calculation

Absorbance corresponds to 0.2 ml of the test = x mg of glucose

100 ml of the sample solution contains =

(x / 0.2) * 25 mg of glucose = % glucose present (Equation 3.8)

3.5. Mineral Analysis

Mineral analysis of Calcium, Zinc, Iron and Magnesium was determined by AOAC (2001) 968.08 using Atomic Absorption Spectroscopy. Five gram of flour sample from was weighed into crucible and dried in an oven at 105 ^{O}C and then the dried samples were placed in to the muffle furnace at 550 ^{0}C for 16 hrs in order to remove the carbonaceous material and cool the crucible with ash in a desiccator. The ash was transferred into 250 mL beaker and moistened with demineralized water. The crucible was washed with 5mL of 12M HCl and carefully transferred into the beaker. Then 12M HCl was added drop-wise with agitation until all effervescence stopped and then evaporated in hot plate which followed by 15mL of 6M HCl and 120 mL of distilled water was added in to the beaker and gently boiled until no more ash could be seen to dissolve. Aliquot was then filtered on ash-free filter paper and collect the filtrate in a 250 mL volumetric flask. The beaker was washed with 5mL of hot 6M HCl, boiling water and filtered then make up to the mark with demineralized water. The blank was prepared by taking the same amount of reagents through the steps all of the above without the sample. The calibration curve was prepared by plotting the absorption or emission values against the metal concentration that correspond to the absorption or emission values of the samples and the blank. The concentration contents were obtained by the following formula:-

Concentration (mg/100g) = (A-b)* V /10*wt. of sample (Equation 3.9)

Where; A = con. in (µg/ml) of sample solution

b = con. in (µg/ml) of blank solution

V = volume in ml of the extract

3.6. Functional properties

Water Absorption Capacity (WAC)

Water absorption capacity of the samples was determined by using the methods suggested by Sathe *et al*., (1982a). One gram of *Triticum aestivum* L. and *Zea mays* L. flour sample from each year was weighed by analytical balance (Model ARA520, USA) and mixed by vortex (Model VM – 300P, Taiwan) with 10 mL distilled water, allowed to stand at ambient temperature (30 ± 2 ^{0}C) for 30 minutes and centrifuged tubes for 5 minutes on a magnetic stirrer. The mixture was centrifuged (Model, 300D, China) at 3500 rpm for 30 minutes. The sediments were weighed after complete removal of the supernatant. WAC was examined as percent water bound per gram flour and calculated using:-

$$\text{WAC}= \frac{(\text{initial water} - \text{supernatant})}{\text{Weight of sample}} * 100 \qquad \text{(Equation 3.10)}$$

Oil Absorption Capacity (OAC)

Oil absorption capacity of the samples was determined by using the methods suggested by Adeleke *et al*., (2010). One gram of flour from each sample was weighed by analytical balance (Model ARA520, USA) and mixed by vortex (Model VM – 300P, Taiwan) with 10 mL of vegetable oil of a known density (0.92 g/ml), allowed to stand at ambient temperature (30 ± 2 ^{0}C) for 30 minutes and centrifuged (Model, 300D, China) at 4000 rpm for 20 minutes. The supernatant removed and measured with 10 mL measuring cylinder and the difference in volume is the oil absorbed by the sample. OAC was calculated using:-

OAC (mL) = (Volume of the added oil – Volume of decanted oil) (Equation 3.11)

Bulk density (BD)

Bulk density of the samples was determined by using the methods suggested by Maninder *et al.*, (2007). Ten gram of *Zea mays* L. and *Triticum aestivum* L. flour samples weighed by analytical balance (Model ARA520, USA) were put into gently filled into 100 ml measuring cylinder. The bottom of each cylinder was tapped gently on a laboratory bench several times until constant volume. Bulk density (g/ml) was then calculated using the formula:

$$BD = \frac{(weight\ of\ sample\ )}{(Volume\ of\ sample)} \qquad \text{(Equation 3.12)}$$

Determination of wet gluten from *Triticum aestivum* L. flour

Wet gluten is an elastic-plastic proteinous substance with two main protein fractions namely gliadins and glutenins, which is obtained after washing out the starch from wheat flour balled dough. Wet gluten determination was done by hand washing method ten gram of bread wheat (*Triticum aestivum* L.) flour was weighed by analytical balance (Model ARA520, USA) and transferred to mixing chamber and 4.8 mL of the 2% sodium chloride solution was added and allowed for 10 minutes in the chamber. Mixing and kneading until a rubbery, soft ball of dough with hand was continued. Washing and working the dough with your hands under running cool distilled water squeeze gently procedures were preceded simultaneously. In this process, starch, water and salt soluble proteins were washed out via murky like water. Then the gluten was press-dried between hands and rolled in to ball. The total weight of the gluten is defined as gluten quantity and wet gluten content of the sample was expressed as a percentage of the mass of the original sample (AACC, 2000). The wet gluten value was derived by the following formula:

$$\text{Wet gluten content (\%)} = \frac{(total\ gluten\ \ )}{10g}*100 = \text{total gluten}*10\text{g} \qquad \text{(Equation 3.13)}$$

Figure: 3.2. Determinations of wet gluten from ball of dough of *Triticum aestivum* L. flour by hand wash method

3. 7. Statistical analysis

Chemical and mineral composition, functional properties and glucose percentage of white maize (*Zea mays* L.) and bread wheat (*Triticum aestivum* L.) data derived from laboratory analysis was statistically analyzed by one way analysis of variance (ANOVA) to see the effect of increasing atmospheric CO_2 on the two samples selected based on their photosynthetic path difference and harvested years with five years intervals (1987, 1992, 1997, 2002, 2007,2012 and 2017 G.C) least significant difference (LSD) was used to compare means among the treatments and significant difference was determined at $P < 0.05$ level. The statistical package used was SPSS version 20.

CHAPTER FOUR

4. Result and Discussion

In this chapter proximate composition and mineral composition, functional properties (WAC, OAC, BD of *Zea mays* L. and *Triticum aestivum* L. and Wet gluten only for *Triticum aestivum* L.) and glucose percentage of the flour samples of the two species grains (*Zea mays* L. and *Triticum aestivum* L.) are discussed. Tables show the results of proximate composition, mineral composition, functional properties and graphs are used for glucose percentage and wet gluten percentage.

4.1. Proximate Composition

From different response of *Zea mays* L. and *Triticum aestivum* L. grains to increasing atmospheric CO_2, change in chemical composition is the primary concern. Proximate composition of White maize (*Zea mays* L.) and Bread wheat (*Triticum aestivum* L.) flour samples is presented in Table 4.1.1 and 4.1.2, respectively.

Table: 4.1.1. Proximate composition of White maize (*Zea mays* L.) flour samples

Composition (g/100g)							
Sample type	**Moisture**	**Crude Fat**	**Crude Protein**	**Total Ash**	**Crude Fiber**	**Utilizable Carbohydrate**	**Gross energy (Kcal)**
87M	5.22±.05^{f}	8.40±.01^{a}	6.25±.01^{a}	1.08±.01^{a}	3.15±00^{a}	81.11±.15^{g}	425.38±.42^{a}
92M	5.18±.03^{f}	8.31±.02^{b}	6.26±.04^{a}	1.06±.00^{a}	3.14±.02^{a}	81.40±.07^{f}	424.75±.21^{b}
97M	5.71±.05^{e}	8.20±.03^{c}	5.46±.03^{b}	1.04±.00^{a}	3.15±.01^{a}	82.14±.15^{e}	424.22±.25^{c}
02M	5.88±.04^{d}	7.62±.05^{d}	5.50±.06^{b}	1.03±.00^{a}	3.13±.01^{a}	82.74±.04^{d}	421.48±.11^{d}
07M	6.09±.03^{c}	7.16±.04^{e}	5.05±.10^{c}	1.05±.00^{a}	3.11±.01^{a}	83.63±.12^{c}	419.14±.21^{e}
12M	6.26±.04^{b}	6.81±.01^{f}	5.10±.04^{c}	1.04 ±.00^{a}	3.11±.01^{a}	84.41±.16^{b}	417.43±.08^{f}
17M	6.42±.02^{a}	6.07±.06^{g}	4.07±.03^{d}	1.04±.00^{a}	3.16±.01^{a}	85.66±.12^{a}	413.51±.15^{g}

All data are reported on dry weight basis, all values were the mean of triplicate ± SD

Means in the same column with different letters are significantly different ($P<0.05$)

Key1: a, b, c, d, e, f ….. are superscripts given to show the significant difference between means

Key2: letters and numbers are represent:-

M87 ………………… Maize sampled from 1987 G.C

M92 ………………… Maize sampled from 1992 G.C

M97 ………………… Maize sampled from 1997 G.C

M02 ………………… Maize sampled from 2002 G.C

M07 ………………… Maize sampled from 2007 G.C

M12 ………………… Maize sampled from 2012 G.C

M17 …………………. Maize sampled from 2017 G.C

Table: 4.1.2. Proximate composition of Bread wheat (*Triticum aestivum* L.) flour samples

Composition (g/100g)							
Sample type	**Moisture**	**Crude Fat**	**Crude Protein**	**Total Ash**	**Crude Fiber**	**Utilizable Carbohydrate**	**Gross energy (KCal**
87W	3.21±.06^{d}	3.70±.06^{a}	14.13±.01^{a}	0.78±.02^{e}	1.41±.01^{b}	79.91±.11^{g}	409.44±.28^{a}
92W	3.17±.03^{d}	3.32±.05^{b}	13.88±.02^{a}	0.84±.00^{d}	1.45±.01^{b}	80.46±.16^{f}	407.21±.50^{b}
97W	3.50±.02^{c}	2.04±.04^{c}	12.23±.08^{b}	0.91±.01^{bc}	1.43±.01^{b}	83.41±.43^{e}	400.82±.14^{c}
02W	3.54±.05^{c}	1.88±.05^{d}	10.42±.03^{c}	0.94±.01^{b}	1.42±.02^{b}	85.39±.43^{d}	400.06±.20^{d}
07W	4.07±.06^{b}	1.60±.01^{e}	9.84±.07^{d}	0.99±.02^{b}	1.44±.00^{b}	86.15±.52^{c}	398.26±.47^{e}
12W	4.12±.10^{b}	1.51±.05^{f}	9.01±.21^{e}	1.08±.00^{a}	1.40±.02^{b}	87.02±.51^{b}	397.60±.20^{f}
17W	4.34±.02^{a}	1.43±.03^{g}	8.58±.40^{f}	1.06±.01^{a}	1.46±00^{b}	87.45±.48^{a}	396.96±.12^{g}

All data are reported on dry weight basis, all values were the mean of triplicate ± SD

Means in the same column with different letters are significantly different ($P<0.05$)

Key1: a, b, c, d, e, f ….. are superscripts given to show the significant difference between means

Key2: letters and numbers are represent:-

W87 …………………Wheat sampled from 1987 G.C

W92 …………...…… Wheat sampled from 1992 G.C

W97 ……………...… Wheat sampled from 1997 G.C

W02 …………...…… Wheat sampled from 2002 G.C

W07 …………...…… Wheat sampled from 2007 G.C

W12 …………...…… Wheat sampled from 2012 G.C

W17 ……………..… Wheat sampled from 2017 G.C

4.1.1. Moisture Content

According to (Table 4.1.1) the moisture content of *Zea mays* L. was ranges from 5.18% to 6.42% and in case of *Triticum aestivum* L. samples on (Table 4.1.2) the moisture content ranges from 3.17% to 4.34% in the past thirty years. In both samples of *Triticum aestivum* L. and *Zea mays* L. from 1987 G.C to 2017 G.C the moisture content shows a significance ($P<0.05$) increment and this increment in both types of samples might be due to decrement in the photorespiration and increment in water use efficiency at elevating atmospheric CO_2 which in turn enhance the moisture content in crop grains due to less loss of water (Fabio *et al*., 2010; Ainsworth and Long, 2005; Wang and Frei, 2011)

4.1.2. Crude Fat

As shown in (Table 4.1.1 and 4.1.2), the crude fat contents in *Zea mays* L. and *Triticum aestivum* L. ranges from 8.40% to 6.07% and 3.70% to 1.43% respectively. The maximum fat content obtained in both samples was from 1987 G.C before thirty years from now. Based on the result from the statistical package used in this study, the decrement were significant ($P<0.05$) in crude fat contents of white maize (*Zea mays* L.) and bread wheat (*Triticum aestivum* L.) flour samples. Since fat content is more dependent on elevating temperature which is a possible result of increasing atmospheric CO_2, that had an effect on reducing of the amounts of accumulated lipids of crops grains (Fabio *et al*., 2010). Therefore, reason for the decrement of crude fat content in both *Zea mays* L. and *Triticum aestivum* L. samples might be due to the increasing of temperature as a result of increasing atmospheric CO_2 in the past three decades.

4.1.3. Crude Protein

The value of crude protein in *Zea mays* L. ranges from 6.25% to 4.07% and on the other hand crude protein content of *Triticum aestivum* L. from 14.13% to 8.58%. Based on these values, there was significant ($P<0.05$) decrement of protein content. The decrement and variation in crude protein content decrement of *Triticum aestivum* L. and *Zea mays* L. might be due to their response and difference in their carbon fixation photosynthetic pathways, since increasing atmospheric CO_2 could enhance concentration of carbohydrates and thus reduce the concentrations of other constituents of the crop, often referred to as the dilution (Gifford *et al*., 2000; Wu *et al*., 2004; Ainsworth and Long, 2005). Eventhough photosynthetic Nitrogen use efficiency can potentially increase under increase atmospheric CO_2 and consequently more carbon can be assimilated with the same or more than the amount of N, resulting in a relative decrement in N content in the leaf. Small amount of organic N in the soil exists as a soluble form to plants to use, both the organic as well as synthetic N fertilizer need to convert to NO^-_3 (nitrate) via nitrification and easily moves toward plants root as they absorb water but as atmospheric CO_2 elevate water absorption of plants also reduced. This readily absorbable nitrate is easily leachable also therefore, due to the increment of relative humidity, moisture and total water content in the soil this essential nutrient might get to be leached (Leakey *et al*., 2009). The other possible effect of atmospheric CO_2 on reduction of grains might be that, all most all crop plants take or need N from the soil continuously via out their lives; which means that N demand enhance as a crop plant size increase due to enhancement of net photosynthesis as a result of CO_2 elevation (Nimesha *et al*., 2012).

Most of the grain N is translocated from parts of the plant like leaf during grain filling; grain N content could also be affected under increased atmospheric CO_2 which has a powerful negative impact on composition of protein content (Seneweera *et al*., 2005). Similar finding were reported by Blumenthal *et al*., (1996); Petra *et al*., (2017); Wieser *et al*., (2008); Wieser *et al*., (2009), Taub, (2010); those was done on comparing flour protein quality and quantity and which are supports the hypothesis of this study that increasing atmospheric CO_2 might reduce protein content highly for C_3 than C_4 crops.

4.1.4. Total Ash

Crude ash value of *Zea mays* L. and *Triticum aestivum* L. samples ranges from 1.08% to 1.03% and 0.78% to 1.06% (Table 4.1.1 and 4.1.2). There was no significant ($P>0.05$) difference in crude ash content of *Zea mays* L. and on the other hand *Triticum aestivum* L. flour samples in the past three decades with five years interval there was a significance ($P<0.05$) increment. This increment in crude ash content of *Triticum aestivum* L. might be due to the possible effect of elevating atmospheric CO_2 prolong the ripening or maturity time of the grains which in turn lead grains to have a stronger or hard bran as well as reducing the digestibility of their fiber content most likely in C_3 crops like *Triticum aestivum* L. (Leakey *et al*., 2009).

4.1.5. Crude Fiber

Crude fiber content of *Zea mays* L. ranges from 3.16% to 3.11% in the past thirty years and bread wheat (*Triticum aestivum* L.) from 1.41% to 1.46% and based on the results on table 4.1 there was no significant difference ($P>0.05$) on fiber content of *Zea mays* L. and *Triticum aestivum* L. flour samples.

4.1.6. Utilizable Carbohydrate

Utilizable carbohydrate content in this study was determined by difference. According to carbohydrate values shows on (Table 4.1.1) for *Zea mays* L. carbohydrate content ranged from 81.11% to 85.66% and (Table 4.1.2) in case of *Triticum aestivum* L. Utilizable carbohydrate was ranged from 79.91% to 87.45%. There were significant ($P<0.05$) increment in both samples of carbohydrate content. The minimum carbohydrate value obtained in samples from 1987 G.C and the maximum on 2017 G.C in the two samples from the past thirty years. The increment of carbohydrate in key crop grains as a result of elevating CO_2 in the atmosphere was discussed in many studies. This increment could be due to the accumulation of carbohydrates as a result of the enhancement in Carbon allocation in all parts of the crops including the grains because of increment of atmospheric CO_2 especially for crop grains with C_3 photosynthetic pathway and slightly for (*Zea mays* L.) which is a C_4 photosynthetic pathway crop. Change in carbohydrate content might have a negative impact for the future in human health by leading to different diet related disorders and diseases (Petra *et al*., 2017; Leon and Vara, 2014; Wieser *et al*., 2009).

Table: 4.1.3. Correlation between Utilizable Carbohydrate and protein percentages of C_3 (*Triticum aestivum* L.) and C_4 (*Zea mays* L.) samples from the past three decades

		Utilizable Carbohydrate	Protein
C_3 Utilizable Carbohydrate	Pearson Correlation	1	-.995**
	Sig. (2-tailed)		.000
C_3 Protein	Pearson Correlation	-.995**	1
	Sig. (2-tailed)	.000	
C_4 Utilizable Carbohydrate	Pearson Correlation	1	-.962**
	Sig. (2-tailed)		.001
C_4 Protein	Pearson Correlation	-.962**	1
	Sig. (2-tailed)	.001	

** Correlation is significant at the 0.05 level (2-tailed).

Where: C_3- three carbon fixation photosynthetic pathway crop (*Triticum aestivum* L.), C_4 -four carbon fixation photosynthetic pathway crop (*Zea mays* L.)

In general the decrements in protein content and variation in the decrement between the two different carbon fixation pathway crop grains could be due to their response and difference in their carbon fixation photosynthetic pathways. increasing of carbohydrates as a result of elevating atmospheric CO_2 which in turn could reduce the concentrations of other constituents of the crop like protein via dilution. Poor absorption and transportation is also the other cause to reduction of grain proteins grown at increasing atmospheric CO_2 (Table 4.1.1 and 4.1.2). This correlation was expressed in terms of increased utilizable carbohydrate in both samples in the past thirty years. Statistically a Pearson correlation coefficient of r = -0.995 and r = -0.962 was calculated for the correlation between utilizable carbohydrate and protein content of *Triticum aestivum* L. and *Zea mays* L. samples.

4.1.7. Total energy

The above tables (4.1.1 and 4.1.2) which represent the total energy content of white maize (*Zea mays* L.) and bread wheat (*Triticum aestivum* L.), respectively. The total energy ranges from 425.38 KCal/100gm to 413.51 KCal/100gm and 409.44 KCal/100gm to 396.96 KCal/100gm, in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.), respectively. Based on this results there was a significant ($P<0.05$) decrement in total energy content of both samples in this study. The maximum value was recorded from in the first sampled year (1987 G.C) and the minimum in (2017 G.C) thirty years after in both samples. The decrement in total energy might be due to the differences in the other components of the samples (i.e. protein, fat, carbohydrate). It has been described that CO_2 could enhance concentration of carbohydrates, starch and simple sugars being the major components, and thus reduce the concentrations of other constituents like fat and protein which are the common components of energy as a result of nutrient dilution which may leads to decrement in total energy gross as well for the future (Johan and Kanplei, 2012; Malin *et al*.,2017).

4.2. Total Glucose

In this study based on (Figure 4.1), the values of total glucose of *Zea mays* L. and *Triticum aestivum* L. range from 58.68% to 80.43% and 55.8% to 84.95%, for the two samples respectively. There was an increment in glucose value of white maize (*Zea mays* L.) and bread wheat (*Triticum aestivum* L.) flour samples which has a significant ($P<0.05$) difference. The minimum value in both samples was observed in the sample from 1987 G.C and the maximum in 2017 G.C. as a year's increase with five years intervals increment in total glucose content was seen as well. It's stated that the increment of atmospheric CO_2 is able to lead the accumulation of carbohydrates to increase on average by 30%–45% or more (Wieser *et al*., 2009). Therefore, this increment might be exhibit due to the increment in carbohydrate accumulation in the two samples, as a result of increasing atmospheric CO_2 from year to years (Leon and Vara, 2014; Leakey *et al*., 2009 and Gifford *et al*., 2000).

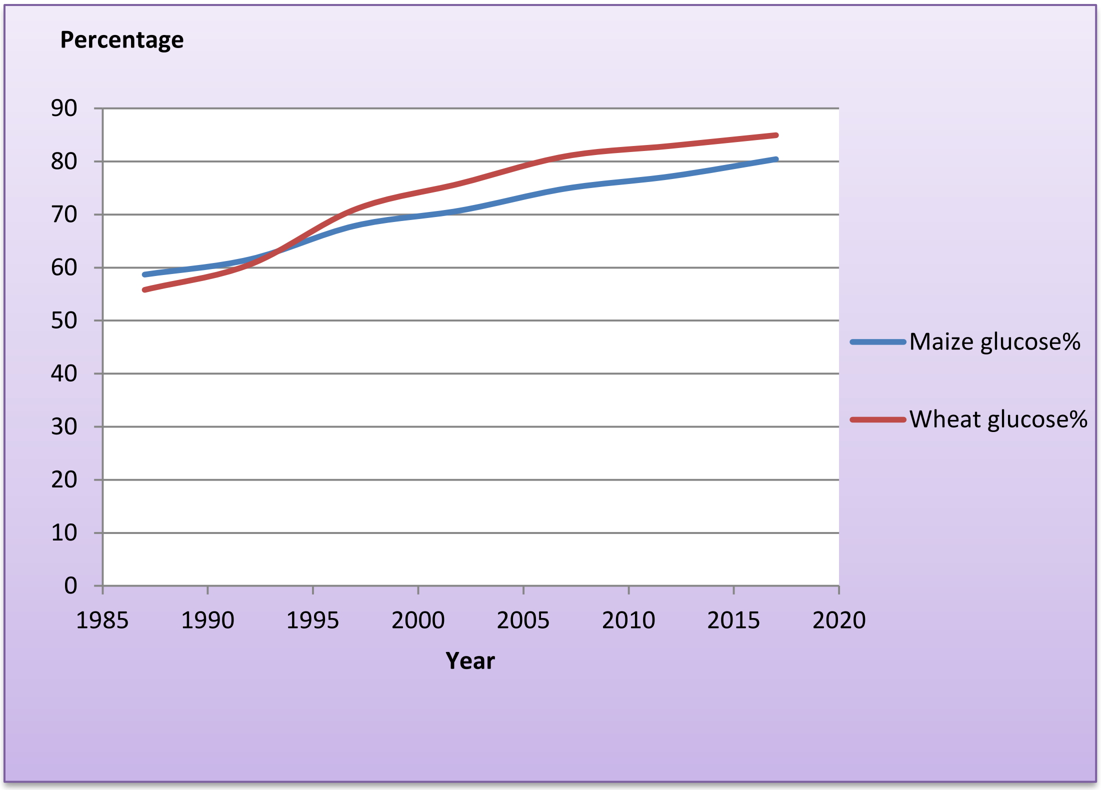

Figure: 4.1.Total glucose in *Zea mays* L. and *Triticum aestivum* L. from 1987 G.C to 2017 G.C

4.3. Minerals

The mineral levels of the two samples white maize (*Zea mays* L.) and bread wheat (*Triticum aestivum* L.) flour are presented on (Table 4.1.4 and 4.1.5), respectively and the analyzed minerals were (Fe, Zn, Ca and Mg).

Table: 4.1.4. Mineral contents of white maize (*Zea mays* L.)

	Composition (mg/g)			
Sample type	**Calcium**	**Magnesium**	**Zinc**	**Iron**
87M	$22.05\pm.04^{a}$	$120.07\pm.08^{a}$	$2.02\pm.02^{a}$	$1.97\pm.02^{a}$
92M	$21.80\pm.05^{b}$	$120.10\pm.02^{a}$	$2.01\pm.01^{a}$	$1.99\pm.01^{a}$
97M	$19.93\pm.06^{c}$	$120.01\pm.10^{b}$	$2.00\pm.01^{a}$	$1.90\pm.00^{b}$
02M	$19.97\pm.03^{c}$	$117.35\pm.26^{c}$	$1.95\pm.01^{a}$	$1.91\pm.00^{b}$
07M	$17.26\pm.00^{d}$	$112.08\pm.15^{d}$	$1.99\pm.01^{a}$	$1.93\pm.00^{b}$
12M	$15.97\pm.03^{e}$	$110.06\pm.05^{e}$	$1.88\pm.01^{b}$	$1.84\pm.00^{c}$
17M	$15.99\pm.07^{e}$	$110.01\pm.01^{e}$	$1.80\pm.01^{c}$	$1.87\pm.00^{c}$

All data are reported on dry weight basis, All values were the mean of triplicate ± SD, Means in the same column with different letters are significantly different ($P<0.05$) , Key1: a, b, c, d, e…..etc superscripts given to show the significant difference between means

Key2: letters and numbers are represent:-

M87 …………………Maize sampled from 1987 G.C

M92 ………………… Maize sampled from 1992 G.C

M97 ………………… Maize sampled from 1997 G.C

M02 ………………… Maize sampled from 2002 G.C

M07 ………………… Maize sampled from 2007 G.C

M12 …………………Maize sampled from 2012 G.C

M17 ………………… Maize sampled from 2017 G.C

Table: 4.1.5. Mineral contents of Bread wheat (*Triticum aestivum* L.)

	Composition (mg/g)			
Sample type	**Calcium**	**Magnesium**	**Zinc**	**Iron**
87W	31.50±.31^{a}	70.01±.01^{a}	1.49±.01^{a}	2.20±.04^{a}
92W	31.30±.26^{b}	69.30±.24^{b}	1.30±.01^{b}	2.12±.05^{b}
97W	30.91±.17^{c}	68.93±.11^{c}	1.22±.02^{c}	2.02±.02^{c}
02W	30.24±.40^{d}	65.65±.39^{d}	1.18±.00^{d}	1.80±.05^{d}
07W	30.17±.22^{e}	64.89±.16^{e}	1.10±.02^{e}	1.62±.05^{e}
12W	29.95±.05^{f}	63.90±.02^{f}	1.03±.02^{f}	1.24±.07^{f}
17W	29.09±.07^{g}	60.96±.05^{g}	0.95±.02^{g}	1.03±.03^{g}

All data are reported on dry weight basis, All values were the mean of triplicate ± SD, Means in the same column with different letters are significantly different ($P<0.05$) , Key1: a, b, c, d, e…..etc superscripts given to show the significant difference between means

Key2: letters and numbers are represent:

W87 ………..Wheat sampled from 1987 G.C

W92 ……….. Wheat sampled from 1992 G.C

W97 ……….. Wheat sampled from 1997 G.C

W02 ……….. Wheat sampled from 2002 G.C

W07 ………. Wheat sampled from 2007 G.C

W12 ……….. Wheat sampled from 2012 G.C

W17……….. Wheat sampled from 2017 G.C

4.3.1. Calcium

The values of calcium was presents in (Table 4.1.4 and 4.1.5) for *Zea mays* L. and *Triticum aestivum* L., the calcium content was ranged from 22.05 **(**100mg/g) to 15.99 (100mg/g) and 31.50 **(**100mg/g) to 29.09 **(**100mg/g) in dry weight for the two samples, respectively. The maximum value in both samples was recorded in 1987 G.C and the minimum values in the last years 2012 G.C and 2017 G.C. The decrement to this mineral might be due to the possible effect of increasing CO$_2$ in atmosphere and it's stated that an increasing of this GHG in atmosphere enhances the growth of crops which in turn will need an increased supply of essential plant nutrients in the soil. And even if nutrients supply is good this environmental change could increase C allocations this situation affect the uptake of Ca from the soil and also lead the dilution of this mineral which in turn affect the Ca content of grain which is a common reason for the decrement of most mineral basically in C$_3$ than C$_4$ crops (Jian *et al*, 2015, Fabio *et al*., 2010).

4.3.2. Magnesium

Magnesium content of *Zea mays* L. and *Triticum aestivum* L. flour sample was ranged from 120.10 to 110.01 (100mg/g) and 70.01 to 60.96 (100mg/g). Based on the results on (Table 4.1.4 and 4.1.5), magnesium content of both samples has significant ($P<0.05$) decrement. The reduction in Mg content of white maize was observed ten years after the first sampled year (1987 G.C) and in case of bread wheat the decrements were observed in every five years intervals. This might be due to the ability of increasing atmospheric CO_2 to suppress nutrient uptake or poor transport from the soil to the crop starting from the vegetation and also the dilution of this mineral as a result of enhancing carbohydrate (Stoltzfus *et al*., 2004; Malin, 2015; Fabio *et al*., 2010).

4.3.3. Zinc

Zinc content of white maize (*Zea mays* L.) was from 2.02 (100mg/g) to 1.80 (100mg/g) and bread wheat (*Triticum aestivum* L.) from 1.49 (100mg/g) to 0.95 (100mg/g) which are represent on (Table 4.1.4 and 4.1.5) for two samples, respectively. There was a significant ($P<0.05$) decrement in both samples from the past three decades. The reduction of this essential mineral was slight (observed twenty years later in 2012 G.C from the first sampled year 1987 G.C) in white maize which is one of the common C_4 photosynthetic pathway of carbon fixation than C_3 crop grain in this study (bread wheat) and this variation in reduction of Zn in the past three decades could be due to the difference of their response to elevating CO_2 in the atmosphere as a result of their different carbon fixation processes.

Overall decrement of this essential mineral in these key non-leguminous grains in this study might be due to either the suppression of nutrient uptake or poor transport from the soil or by lower allocation of this mineral during grain filling stage at elevating atmospheric CO_2. The other thing set as a cause to this decrement is the dilution of minerals by allocation of sugar as a result of change in concentration of this GHG in the atmosphere. The same reduction due to elevating atmospheric CO_2 was also observed in studies done under artificial controlled environment (Stoltzfus *et al*., 2004, Nimesha *et al*., 2012; Myers, *et al*., 2014; Malin, 2015 and Fabio *et al*., 2010).

4.3.4. Iron

According to (Table 4.1.4), Iron content of *Zea mays* L. was ranged from 1.99 (100mg/g) to 1.84 (100mg/g) and in case of *Triticum aestivum* L. from 2.20 (100mg/g) to 1.03 (100mg/g), (Table 4.1.5). There was a significant ($P<0.05$) decrement in Fe content of both samples in the past thirty years. Reduction of this mineral in white maize was observed ten years later in (1997 G.C) from the first sampled year (1987 G.C) but in case of bread wheat the change was recorded in every five years intervals this variation could be due to the difference in their carbon fixation. Reduction of this mineral might be due to dilution process as a result of high carbon allocation that acts to reduce mineral concentration and also poor uptake or transport from the soil which is the major causes to the decrement of minerals in crops (Petra *et al*., 2017, Malin, 2015, Fabio *et al*., 2010).

Table: 4.1.6. Correlation between Utilizable Carbohydrate and mineral contents of C_3 (*Triticum aestivum* L.) and C_4 (*Zea mays* L.) samples from the past three decades

		Utilizable Carbohydrate	Calcium	Magnesium	Zinc	Iron
C_3 Utilizable Carbohydrate	Pearson Correlation	1	-.944**	-.926**	-.946**	-.906**
	Sig. (2-tailed)		.001	.003	.001	.005
C_4 Utilizable Carbohydrate	Pearson Correlation	1	-.961**	-.945**	-.918**	-.812**
	Sig. (2-tailed)		.001	.001	.003	.020

** Correlation is significant at the 0.05 level (2-tailed).

Where: C_3- three carbon fixation photosynthetic pathway crop (*Triticum aestivum* L.), C_4 - four carbon fixation photosynthetic pathway crop (*Zea mays* L.)

Mineral contents of *Triticum aestivum* L. and *Zea mays* L. samples in the past three decades with in five year intervals shows a decrement but more notable in C_3 carbon fixation pathway than the C_4 crop grain, which might be due to the suppression of nutrient uptake or poor transport from the soil or by lower allocation and also a high dilution of minerals to the cost of carbohydrate increment at elevating atmospheric CO_2 (Table: 4.1.4 and 4.1.5). The correlation analysis results shown in Table 4.1.6 as Pearson correlation coefficients (r), utilizable Carbohydrate of *Triticum aestivum* L. and *Zea mays* L. values correlated to mineral contents (Ca, Mg, Zn and Fe) of *Triticum aestivum* L. and *Zea mays* L. values at $r = -0.944$, $r = -0.926$, $r = -0.946$ and $r = -0.906$, and r = -0.961, r = -0.945, r = -0.918 and r = -0.812 respectively, for the two samples and four minerals (Ca, Mg, Zn and Fe).

4.4. Functional properties

Functional properties govern the suitability of protein and carbohydrate either as food supplement or as ingredient for fabricating new food products. Since wheat and maize are the most common grains used for different industrial purposes to a large extent, alteration in quality may affect the market value and quality of products. Many measures of functional properties are related to the content and quality of protein, starch and others, such as gluten composition, dough elasticity/resistance, and bread loaf volume, and consequently these variables are likely to be impaired by increment of atmospheric CO_2 following the pattern of grain N concentration and increasing of carbohydrate majorly (Leakey *et al*., 2009). Therefore, it is relevant to study how different functional properties affected by increasing atmospheric CO_2 in the past thirty years. The Water absorption capacity, Oil absorption capacity, Bulk density of white maize (*Zea mays* L.) and bread wheat (*Triticum aestivum* L.), and Wet gluten (%) for *Triticum aestivum* L. were determined.

Table: 4.1.7. Functional properties of white maize (*Zea mays* L.) flour

Sample type	**WAC (ml/g)**	**OAC (ml/g)**	**BD (g/ml)**
M87	196.03±.50^{g}	1.76±.02^{a}	0.85±.00^{c}
M92	196±.06^{f}	1.72±.02^{a}	0.90±.00^{c}
M97	196.66±.40^{e}	1.65±.01^{b}	0.97±.00^{b}
M02	198.89±.51^{d}	1.60±.02^{c}	0.98±.01^{b}
M07	198.56±.60^{c}	1.52±.01^{d}	0.98±.05^{b}
M12	199.90±.08^{b}	1.40±.05^{e}	1.02±.01^{a}
M17	210±.05^{a}	1.43±.05^{e}	1.07±.00^{a}

All data are reported on dry weight basis, all values were the mean of triplicate ± SD, Means in the same column with different letters are significantly different (P<0.05, Key1: a, b, c, d, e…..etc superscripts given to show the significant difference between means, Where: WAC = Water Absorption Capacity, OAC = Oil Absorption Capacity and BD = Bulk Density

M87 …………………Maize sampled from 1987 G.C

M92 ………………… Maize sampled from 1992 G.C

M97 ………………… Maize sampled from 1997 G.C

M02 ………………… Maize sampled from 2002 G.C

M07 ………………… Maize sampled from 2007 G.C

M12 ………………….Maize sampled from 2012 G.C

M17 ………………… Maize sampled from 2017 G.C

Table: 4.1.8. Functional properties of bread wheat (*Triticum aestivum* L.) flour

Sample type	**WAC (ml/g)**	**OAC (ml/g)**	**BD (g/ml)**
W87	171±.60^{g}	1.95±.03^{a}	0.58±.01^{e}
W92	173.03±.71^{f}	1.80±.04^{b}	0.64±.00^{d}
W97	179.07±.10^{e}	1.71±.05^{c}	0.61±.00^{d}
W02	180.6±.07^{d}	1.62±.06^{d}	0.72±.01^{c}
W07	184.90±.09^{c}	1.53±.07^{e}	0.80±.01^{b}
W12	189.23±.50^{b}	1.34±.06^{f}	0.87±.02^{a}
W17	191.16±.06^{a}	1.27±.04^{g}	0.89±.00^{a}

All data are reported on dry weight basis, all values were the mean of triplicate ± SD, Means in the same column with different letters are significantly different ($P<0.05$), Key1: a, b, c, d, e…..etc superscripts given to show the significant difference between means

Where: WAC = Water Absorption Capacity, OAC = Oil Absorption Capacity and BD = Bulk Density

W87 ………..Wheat sampled from 1987 G.C

W92 ……….. Wheat sampled from 1992 G.C

W97 ……….. Wheat sampled from 1997 G.C

W02 ……….. Wheat sampled from 2002 G.C

W07 ………. Wheat sampled from 2007 G.C

W12 ……….. Wheat sampled from 2012 G.C

W17……….. Wheat sampled from 2017 G.C

4.4.1. Water absorption capacity

Water absorption capacity (WAC) of white maize (*Zea mays* L.) were shown in (Table 4.1.7), the values were ranged from 196 (ml/g) to 210 (ml/g) and in the past three decades with five years interval, in case of bread wheat (*Triticum aestivum* L.) in (Table 4.1.8) this functional property values ranged from 171 (ml/g) to 191.16 (ml/g). Both the samples in this study shows a significant ($P<0.05$) increment in WAC. Since WAC is one of the functional property to represent characteristics or ability of the flour to associate with water under conditions when water is limiting and it is important in the development of ready to eat foods, This property determines the hydrophilic nature and high hydrogen bonding of protein molecules in the grain flour, also the enhancement of this functional property of grains flour could be a result of the presence of higher amount of carbohydrates and fibre in the flour (Adebowale *et al*, 2005; Muhammad *et al*., 2013). Therefore, this increment in WAC of both samples in this study might be due to changes occurred as a result of increasing atmospheric CO_2 that might be responsible to decrement of protein content as a result of the dilution by increasing of carbohydrate (sugars) in both *Zea mays* L. and *Triticum aestivum* L. samples in the past thirty years.

4.4.2. Oil absorption capacity

The results of Oil absorption capacity (OAC) of *Zea mays* L. and *Triticum aestivum* L. samples in the past three decades were shown in (Table 4.1.7 and 4.1.8), respectively. Results ranged from 1.76 (ml/g) to 1.40 (ml/g) and 1.95 (ml/g) to 1.27 (ml/g) in *Zea mays* L. and *Triticum aestivum* L., respectively. There was a significant ($P<0.05$) decrement of OAC in the past three decades with five years intervals in both samples. Therefore, the reduction of this functional property could be due to a noticeable reduction of crude protein which might be as a result of the possible effect of increasing atmospheric CO_2 that in turn cause the dilution of protein and also lead the crop to be less efficient to the absorbance of NO^-_3 (readily absorbable form of N component formed after nitrification process) from the soil, which in turn affect the scarcity of protein to the grain filling stage (Wieser *et al*., 2008; Wieser *et al*., 2009; Taub, 2010). OAC is another important functional property since it plays a great role in enhancing the mouth feel while retaining the flavor of food products; it is attributed mainly to the physical entrapment of oils and an indication of the rate at which protein binds to fat in food formulations (Malomo *et al*, 2012; Onimawo and Akubor, 2012). It indicates the enhanced hydrophobic character of proteins in the flour; it is exhibited by the proteins in the flour which physically bind to fat by capillary attraction (Taub*et al*, 2008, Shewry and Halford, 2002).

4.4.3. Bulk density

The values of the Bulk density (BD) for *Zea mays* L. and *Triticum aestivum* L. samples were represent in (Table 4.1.5 and 4.1.6), in the past three decades BD of the two samples ranged from 0.85 (g/ml) to 1.07(g/ml) and 0.58 (g/ml) to 0.89 (g/ml), respectively. There was a significant ($P<0.05$) increment in BD of *Zea mays* L. and *Triticum aestivum* L. The increment in BD of in both samples but slight in white maize than bread wheat might be due to either the difference in their carbon fixation processes or due to possible increment in the carbohydrate (glucose) content of both samples as a result of elevating CO_2 in the atmosphere which in turn could have an effect on structure of starch polymers as well. An extra glucose in plants stored as a starch that the plant can convert it back to glucose when it's needed and increasing of atmospheric CO_2 cause plants to produce an extra glucose, which might be lead to changes in either structure or amount of starch in the grains. It has been reported by (Awolu *et al*, 2017) that BD is influenced by the loose in structure of the starch polymers, could result in low bulk density, being proteinous in nature does not significantly contribute to BD. And based on this report the Higher BD is desirable for greater ease of dispersibility, packaging and reduction of paste thickness but which is not suitable for determining transportation and storability.

4.4.4. Determination of wet gluten from *Triticum aestivum* L. flour

In this study wet gluten content of *Triticum aestivum* L was also determined via hand washing method and as shown in (Figure 4.2), the values of wet gluten was in the seven bread wheat samples ranged from 23.92 % to 22.4% in the past three decades with five years intervals. There was a significant ($P<0.05$) decrement in gluten (gliadin and glutenin) proteins which are representing the major protein fraction of the starchy endosperm and play a key role in determining the unique baking quality of wheat by conferring cohesivity, viscosity and elasticity on dough.

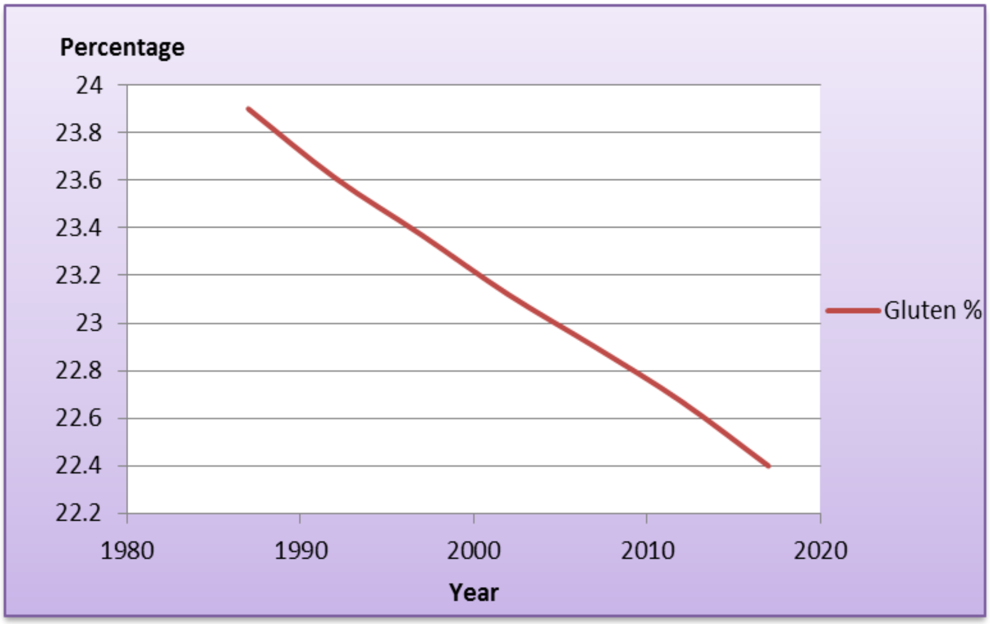

Figure: 4.2.Wet gluten of bread wheat (*Triticum aestivum* L.) flour

This decrement might be due to loose or decrement of a protein content or presence of higher amount of carbohydrates which possibly could be due to common influence of change of CO_2 in the atmosphere (Wieser *et al*., 2008, Wieser *et al*., 2009, Petra *et al*., 2017). Gluten is the protein molecule found in grains including wheat, barley, rye and oats, which is a very beneficial protein which provides elasticity, texture, flavour and helps to retain moisture (Wieser, 2007). Bread is commonly made of wheat flour, meaning that wheat is a prime source of gluten which is a key component form from two proteins gliadin and glutenin those are attach to each other to form gluten during dough formation in the presence of water (Veraverbeke and Delcour, 2002). Since the quantity and quality of gluten are considered the most important quality parameter of wheat flour especially in fabricating food products, this study shows how the quality of bread wheat (*Triticum aestivum* L.) become less as a years of sample increase from (1987 to 2017 G.C).

Table: 4.1.9. Correlation between Protein content and wet gluten percentage of C_3 (*Triticum aestivum* L.) samples from the past three decades

		C_3 Protein	Gluten
C_3 Protein	Pearson Correlation	1	.976**
	Sig. (2-tailed)		.000
Gluten	Pearson Correlation	.976**	1
	Sig. (2-tailed)	.000	

** Correlation is significant at the 0.05 level (2-tailed).

Where: C_3- three carbon fixation photosynthetic pathway crop (*Triticum aestivum* L.)

According to figure (4.2), the amount of Wet gluten of bread wheat (*Triticum aestivum* L.) samples from the past thirty years with five years intervals shows a notable decrement which possible relate to the reduction in the protein content of this grains and the above correlation analysis table (1.4.9) shows that the protein content of *Triticum aestivum* L. values correlated to wet gluten contents values at r = 0.976.

CHAPTER FIVE

5. Conclusion and Recommendation

5.1. Conclusion

This study was conducted with the broad intention to point out some of the possible consequences of increasing CO_2 in the atmosphere to the two most staple food grains in our country. Fourteen samples were analyzed with two different species cultivars and photosynthetic pathway C_3 and C_4 namely, *Zea mays* L. and bread *Triticum aestivum* L., from Ambo district Amaro kebele, West Shoa zone of Oromia, from (1987 to 2017 G.C) with five years intervals.

Chemical composition (proximate and mineral contents), different functional properties including wet gluten and total glucose percentage were analyzed. The moisture content of both samples shows an increment which could lead the grains in the future to be easily infested and damaged by creating a favorable condition to insects and funguses. Notable reduction of protein level on both samples which could be outcome of reduced N concentrations and increment in crude ash content of *Triticum aestivum* L. this would indicate that increment of this GHG possible effect on insolubility of fiber content in wheat. The increment in utilizable carbohydrate and total glucose of both samples, which could be as a result of enhancement of Carbon allocation in the grain filling stage due to elevating CO_2 in the atmosphere.

Even though, glucose is one of the main carbohydrate sugar and most important sources of fuel for several of vital organs, the increment of this simple sugar is expect to increase more as carbohydrate content increase in the future elevating atmospheric CO_2 as its reaches to 1000 ppm and could result in diet associated diseases.

Reducing of minerals content (Ca, Mg, Zn and Fe) in both samples but more notable in the C_3 photosynthetic pathway representing grain flour *Triticum aestivum* L., recorded in the past three decades which could be due to the suppression of nutrient uptake or poor transport from the soil or by lower allocation at elevating atmospheric CO_2 and this will create a risk to increase numbers of newly deficient of this minerals especially in developing countries for the future.

Additionally functional properties in the study were observed. Increment in water absorption capacity and bulk density and reduction of oil absorption capacity was recorded in both samples; and the other functional property which shows a reduction in this study was wet gluten of bread wheat that could indicate changes in atmospheric CO_2 may affect processing quality especially for *Triticum aestivum* L based products processing industries.

Combining all the results of this study, *Triticum aestivum* L. and *Zea mays* L. samples from 1987 to 2017 G.C to investigate possible consequence of increased atmospheric CO_2 in the past three decades has shown a tremendous reduction in the protein and fat content, mineral content in both samples and wet gluten only in *Triticum aestivum* L. on the other hand increment in utilizable carbohydrate and total glucose content in both samples were recorded, which remarkably predicted by different studies. From these results the possible consequence of increasing atmospheric CO_2 on C_3 photosynthetic pathway crop is more notable than C_4 and since Ethiopia is one of the least developed countries, the possible impact of increment of this GHG in atmosphere on agricultural products for the future may create large burdens of diseases and deficiencies because it’s obvious that large numbers of the populations are receive most of their dietary requirements including minerals from these crops.

5.2. Recommendation

Now a days, Ethiopia is experiencing high rates of urban population growth and the economic vision to achieve middle-income status and it's expect to increase demands for energy intensive infrastructure, industrial sector, followed by transportations to register the largest annual growth in CO_2 gas emission for the future. The impact on changes of nutritional composition of agricultural crop grains was observed in this study which need to be seen as a great deal because the scarcity of dietary protein, minerals and increment in utilizable carbohydrate for the future may lead to malnourishment.

The current study with its own different limitations has investigated the possible consequences of (past and ongoing) increase atmospheric CO_2 on bread wheat (*Triticum aestivum* L.) and white maize (*Zea mays* L.) on chemical composition and functional properties. But the following issues should also be considered in the future based on the outcomes of the current study.

- The current study was done on non-legumes grains the possible impact of increasing atmospheric CO_2 on legumes should be studied.
- Samples from midland or woinadega agro-ecological zone (Ambo) was used to see the possible effects of increasing atmospheric CO_2, samples from others agro-ecological zone of the country's should be studied.
- Though the reduction of protein and fat content of *Triticum aestivum* L. and *Zea mays* L are studied, the change in their amino acid and fatty acid composition of these crops has not been studied yet so, further study on them is recommended.
- Since increment in total carbohydrate was observed in this study, further analysis for starch (amylose/amylopectin) should be carried out.
- Only four mineral contents (Ca, Mg, Zn and Fe) was analyzed in this study to see their changes in the past three decades which could be due to elevating CO_2 in the atmosphere, therefore other minerals those are not studied in this thesis could be study.
- The effect of rising atmospheric CO_2 on grain quality of different crop plants in artificially controlled environment could also be study to block different factors and to predict future impact of this GHG on chemical composition of crops.

- Effect of increasing atmospheric CO_2 on vitamins like Ascorbic Acid and Antioxidant components of C_3 and C_4 crops could be study to understand the impacts changes of this common GHG other than carbohydrate and mineral contents of these common grains.
- It's also recommended by the researcher to encourage public awareness on the effect of CO_2 in the atmosphere and its negative consequence on declining of dietary nutrients in crops as well as its health implications which will be more helpful via out the implementations of mitigation plans by the government.

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Annex 1- Greenhouse Gases emissions in Ethiopia in Gigagram

Year (G.C)	**CH_4**	**N_2O**	**CO_2**	**Total national emission**
1994	37,033.398	14,303.658	26,050.54	25,433.179
1995	38,068.558	13,492.568	72,340.089	124,159.49
1996	39,454.12	14,658.637	50,511.81	104,606.07
1997	59,160.622	19,299.657	51,561.39	130,230.52
1998	59,680.902	19,558.628	53,760.92	133,209.21
1999	59,910.412	19,721.684	54,478.54	134,092.33
2000	58,499.454	18,911.616	105,663.2	183,422.00
2001	60,432.116	19,260.924	122,681.3	202,697.74
2002	66,519.072	22,077.237	113,253.8	201,828.80
2003	65,935.504	21,614.561	116,779.5	204,306.18
2004	67,051.694	23,790.877	66,810.55	157,626.65
2005	53,905.573	22,462.551	68,019.98	144,358.07
2006	55,932.864	23,769.002	68,512.75	148,884.24
2007	62,142.798	26,733.978	71,372.27	160,211.53
2008	63,410.744	27,514.442	85,187.04	176,075.07
2009	65,551.894	27,224.805	80,912.06	173,651.75
2010	68,699.37	29,603.401	88,095.24	186,361.60
2011	71,341.652	25,759.662	111,818.2	208,884.77
2012	71,825.46454	30,500.73633	40,266.83	142,590.49
2013	72,793.82214	30,418.03479	42,948.57	146,160.43

(Source: Initial National Communication of Ethiopia, 2001; Second National Communication of Ethiopia to UNFCCC, 2015)

Annex 2- Monthly report of rainfall pattern of Ambo, West Shoa Zone of Oromia region in the past thirty years

Year (G.C)	Months											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1987	1.6	38.1	128.3	119.8	185.4	275.2	315.9	196.1	151.6	13.6	1.4	12.7
1988	1.9	232.6	118.1	141.9	202.9	267.2	345.1	169.0	171.6	17.3	0.0	28.1
1989	10.1	19.8	159.1	123	177.9	278.3	308.7	171.1	134.5	51.8	0.0	42.7
1990	8.09	114.7	141.2	98.82	99.2	211.2	335.6	197.1	94.8	3.3	0.0	31.1
1991	8.3	28.3	130.5	154.1	112.8	232.0	289.3	187.3	194.2	21.8	0.0	41.2
1992	11.5	58.7	152.4	181.9	121.2	229.1	263.5	169.0	121.3	25.1	0.0	0.0
1993	12.6	35.6	110.5	119.87	59.7	189.3	268.5	215.4	121.3	45.9	1.6	0.0
1994	8.20	0.0	89.8	154.1	93.17	196.8	195.9	297.3	166.8	31.1	1.2	21.2
1995	20.3	12.6	44.8	117.4	122.2	89	206.1	213.7	189.0	11.7	0.0	32.8
1996	23.1	20.07	60.7	31.5	109.6	255.2	221.5	198.6	175.1	38.1	0.0	11.2
1997	28.7	0.0	71.0	83.1	59.7	132.0	132.1	183.4	59.2	69.0	22.1	7.2
1998	74.7	10.4	101	101.76	99.2	166.3	201.3	211.6	165.1	14.8	10.8	1.2
1999	8.40	8.4	69.5	51.5	93.7	109.9	195.9	132.9	131.2	119.9	1.3	0.0
2000	10.0	0.0	44.2	108.7	177.9	113.7	195.8	191.6	149.8	83.7	20.7	14.8
2001	0.0	12.5	18.6	112.9	87.4	96.4	197.9	243.1	110.5	41.8	5.4	11.0
2002	58.7	16.9	55.6	56.3	39.5	178.0	164.2	142.7	40.3	17.9	0.0	17.2
2003	41.7	100.0	28.7	18.7	9.4	267.2	134.2	142.1	185.1	9.3	1.0	0.0
2004	39.2	16.6	16.6	47.5	26.8	137.1	203.6	215.0	138.9	19.0	0.0	8.9
2005	25.2	0.0	23.1	72.3	58.1	166.6	158.0	187.1	98.4	19.0	10.2	0.0
2006	0.0	4.7	29.1	145.8	157.3	102.9	132.8	298.6	76.5	17.9	18.8	0.0
2007	19.3	54.3	83.2	38.9	131.6	178.3	152.6	218.3	111.7	11.0	0.0	10.0
2008	26.9	0.0	11.0	44.8	157.3	91.01	308.0	260.2	84.0	64.8	101.8	2.7
2009	122.4	23.1	13.4	61.2	47.5	311.8	285.7	271.9	65.3	56.1	1.7	41.9
2010	55.9	48.4	1.0	88.0	126.7	166.4	315.7	235.1	116.0	0.0	122.6	24.5
2011	94.3	7.0	10.1	111.5	185.8	328.6	456.4	387.1	224.2	0.0	0.0	0.0
2012	0.3	0.0	3.6	56.5	23.1	142.5	283.1	367.7	173.1	53.2	0.0	18.0
2013	0.0	3.9	18.6	18.2	119.8	267.2	226.3	172.3	73.4	94.9	4.0	0.0
2014	102.8	18.2	11.5	153.8	202.9	138.8	130.1	134.2	91.3	28.2	8.9	4.4
2015	74.3	6.1	10.1	61.2	12.7	358.7	184.7	166.7	103.9	0.0	27.9	2.1
2016	97.0	29.1	9.3	104.4	218.9	158.8	185.7	156.4	234.1	103.8	0.0	4.5
2017	0.0	0.0	11.4	27.5	118.9	89.0	152.3	140.9	129.6	7.6	17.6	25.0

(Source: Ambo Agriculture station NMA, 2017)

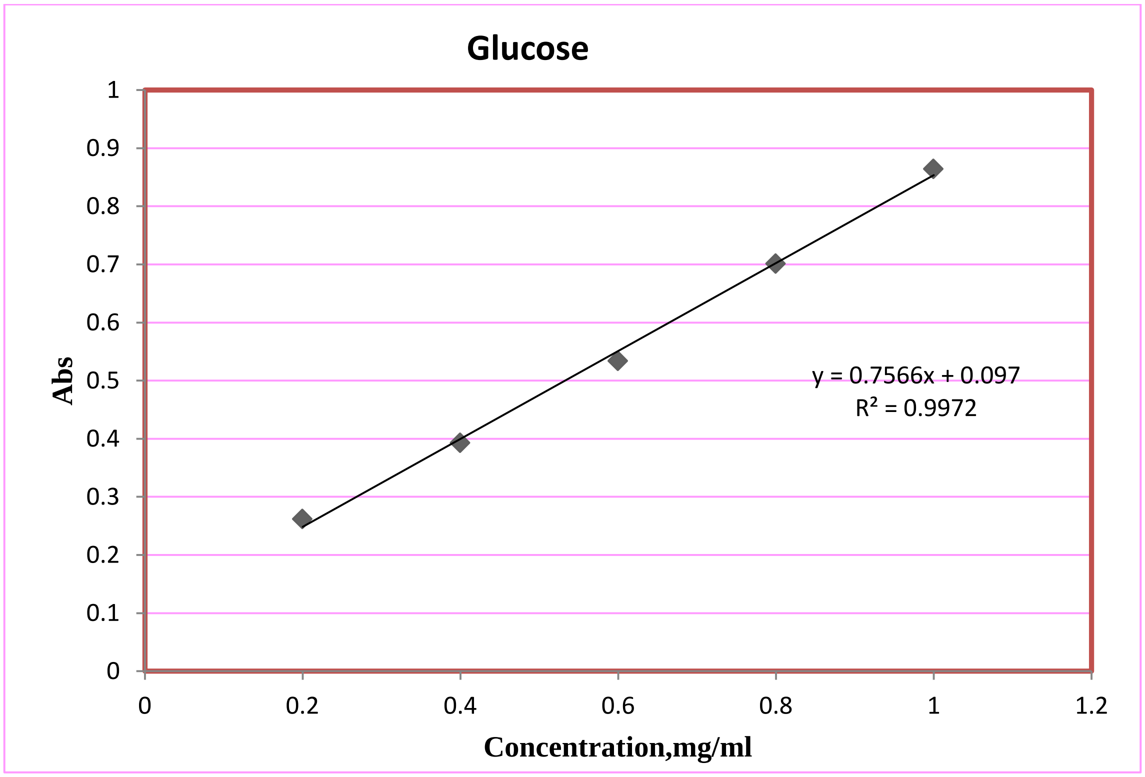
Annex 3- Monthly report of maximum and minimum temperature of Ambo West Shoa Zone of Oromia region in the past thirty years

Year (G.C)		Months											
		Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
1987	max	25.9	26.4	26.6	26.7	26.9	24.3	22.6	22.4	23.8	25.6	24.4	25
	min	11.4	12.9	13.2	13.4	13.5	11.7	8.9	10.8	7.9	10.3	11.4	11.2
1988	max	26.6	25	28.3	27.3	26.2	23.6	21.73	22.6	23.2	24.2	25.6	25.6
	min	11.1	12	13.3	13.2	13.4	11.2	10.4	12.2	10.1	8.4	11.6	11.2
1989	max	25.72	25.7	28	27	26.6	24	22.6	22.6	22.4	22.6	25	25.6
	min	11.7	11.5	11.8	11.8	11.4	11.2	11.7	11.6	8.9	7.8	7.9	12.3
1990	max	26.7	25.3	26.6	27.3	27.5	24.3	22.3	22.1	23.4	25.5	26.5	24.7
	min	11.2	12.4	13.3	12.2	12.3	11.9	11.9	11.9	11.1	9.7	10.6	11.6
1991	max	27.8	27.6	27.3	26.8	27.8	23.2	22.4	20.3	22.1	26.6	25.8	25.4
	min	12.8	12.3	13	12.7	11.1	11.5	11.6	11	10.4	10.6	11.1	11
1992	max	26.2	26.3	26.8	27	26.8	23.6	22.4	22.1	23.3	22.7	25.5	26.3
	min	10.9	10.6	12.7	12.5	12.7	11.6	11.8	11.3	11.2	10.9	11.9	11.4
1993	max	26.7	25.2	28.7	26.8	26.6	25.3	24.4	21.1	24.3	25.7	27.5	26.3
	min	10.6	12	13.2	12.5	12.7	11.6	11.8	11.3	11.2	10.9	11.9	11.4
1994	max	27.8	26.5	29	27.6	28.8	25.1	23.2	22.4	22.1	22.8	25	27.9
	min	11	10.8	13.1	13.4	12.3	11.4	11.4	10.4	10.6	10.1	10.9	10.6
1995	max	27.5	28.4	27.7	28.4	28.7	24	22.7	22.8	23.7	22.1	25.9	24.8
	min	11.5	11.5	13	13.2	11.7	11.8	11.8	11	11.2	9.7	11.4	12.1
1996	max	27.9	25.4	26.5	28	29	25.6	21.4	23.5	21	23.8	27.5	25.3
	min	10.3	10.9	13.6	13.7	11.6	11.5	10.7	11.3	11.5	10.9	11.6	11
1997	max	26.9	27.5	27.3	28.1	26.9	24	23.3	24.3	23.8	24.2	26.7	27.8
	min	10.3	11.9	12.9	13.1	12.3	12	12	11.1	10.1	10.6	11	10.3
1998	max	27.7	29	28.6	30	27.4	24.9	23.5	25.3	26.2	22.9	24.3	25.2
	min	10.8	12.5	13.3	14	11.8	11.6	11.4	11.1	10.1	10.5	8.5	8.7
1999	max	27.3	29.6	28.3	28.7	28	25	21.4	21.9	23.7	24.2	25.3	25.9
	min	9.6	11	12.4	12	10.8	11	12.1	11.4	10.3	10.8	7.8	9.8
2000	max	27	28.3	29.7	27.1	26.8	25.4	22.6	21.9	23.5	24.8	25.3	25.8
	min	11	12	14.2	13.1	11.6	11.1	11.8	11.9	11.5	10.9	7.8	9.7
2001	max	29.6	28.5	26.6	28.1	26.9	23	22.3	22.3	24.3	26.2	25.3	26.7
	min	10.3	11.9	12.9	13.1	12.3	12	12	12.7	9.8	10.3	10.3	10.9
2002	max	27.1	27.6	28.7	28.4	29	25.2	23.9	23.8	24.3	26.5	27.6	28.1
	min	11.5	12.5	11.9	12.7	13	12.1	12.1	11.2	11.5	10.7	11.6	11.5
2003	max	27.4	28.7	28.3	27.4	28.9	24.8	22.3	22.8	23.3	25.7	26.3	26.1
	min	11.3	12.7	13.4	13	11.2	11.8	12.8	11.9	11.2	9.4	11.2	10.4
2004	max	25.7	29	28.1	27.4	28.3	24.9	22.4	22.8	23.6	25.2	26.7	26.1
	min	12.6	12.4	13.9	13.5	11.9	12.2	12	11.9	11.1	10.6	10.4	11.9
2005	max	27	31.1	29.2	28.4	27	25.3	22.4	23.2	24.3	25.7	26.4	26.7
	min	11	12.6	12.9	14.1	12.6	11.3	11.8	11.6	11.5	9.4	9	9
2006	max	27.9	29.2	27.8	26.5	26.5	24.4	22.6	22.2	23.8	25.9	26.3	26.6
	min	11.9	12.6	12.3	13.1	10.9	11.5	12.6	12.7	11.5	11.5	11.8	12.3

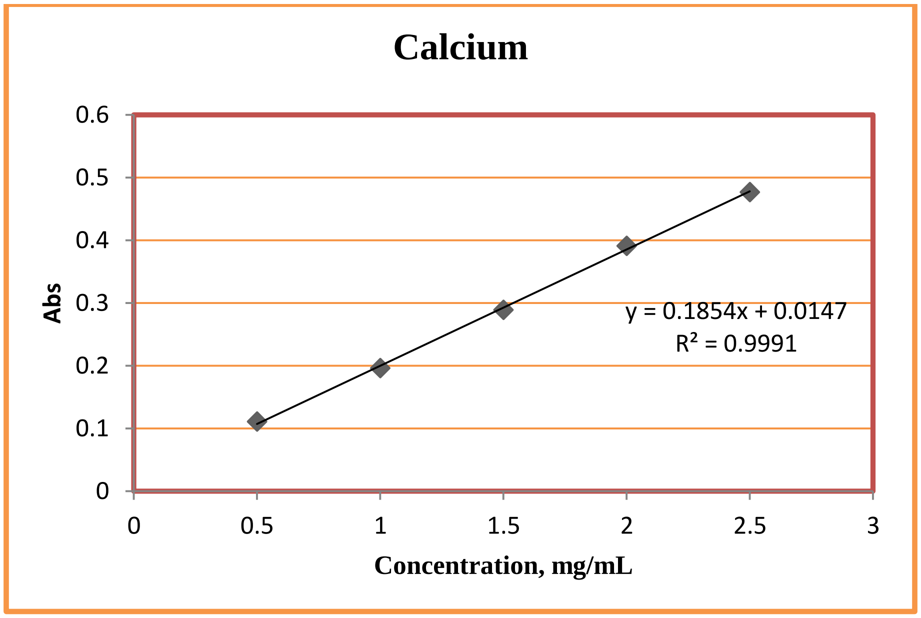
2007	max	27.4	28.2	28.9	28.9	28.9	24.9	22	22.8	24	25.7	27.9	27.8
	min	12.6	11.9	13	13.5	12.5	12	12.6	12.5	12.3	10.1	11.4	11.3
2008	max	28	27.6	30.1	29	26.6	24.3	22.5	22.3	24.2	26	24.9	27.4
	min	12.6	11.9	13.8	14	12.7	11	12.2	11.8	11.3	11.2	11.3	11.6
2009	max	27.2	28.5	29.8	28.7	29,2	27	22.9	23.1	25.5	26.2	27.1	27.6
	min	12.3	13.3	13.9	14.4	13	12.3	12.9	13.1	10.8	11.5	11	12.8
2010	max	28	29.4	28.8	28.8	27.4	23	22.7	22.1	23.9	27.4	27	26.8
	min	12.7	12.4	12.6	14.5	14.1	11.1	13.9	11.8	15.4	14.8	13.4	13.2
2011	max	27.5	27.5	27.8	29.3	27.3	25.2	23.6	23.8	24.4	27	26.3	27.3
	min	13.2	13.2	14.5	15.1	14.9	14.3	13.1	13.1	14.3	13.9	13.5	12.4
2012	max	28.4	28.9	29.1	29.7	29.5	25.7	24.7	24	24.5	26	28.2	28
	min	12.2	12.9	14.1	13.9	13.8	12.2	12.9	12.9	12.1	10.6	11.3	12.8
2013	max	29	29.4	29.7	29.6	26.4	24.7	21.7	21.8	23	25.1	26.2	27.7
	min	12.2	13.3	14.9	13.7	13.3	13.6	12.9	13.1	12.1	11.3	12.3	12.4
2014	max	27.7	26	29	28.2	29.8	25.6	22.4	22.7	24.2	25.7	26.7	27.9
	min	12.9	13.3	13.2	14.1	12	12.8	13.4	12.3	11.6	11.8	11	13
2015	max	27.7	29.3	29.7	30.1	30.7	25.3	24.5	23.7	23.7	27.2	27	27.4
	min	10.6	12.9	13.9	13.7	13.8	13.3	12.8	12.8	12.2	11.5	12	11.7
2016	max	27.8	31.5	30.8	29.3	26.4	23.1	23.7	23.7	21	27.8	26.1	26.3
	min	12.6	13.7	14.7	15.1	13.6	11	12.3	12.6	13.7	12.4	9.8	10.1
2017	max	30.6	29.7	29.9	32	30.1	27.1	29.2	28.3	28.9	27.3	28.9	31.7
	min	9.6	13.8	14	15.2	13.9	12.6	14.1	13.8	13.6	12.7	14	12.6

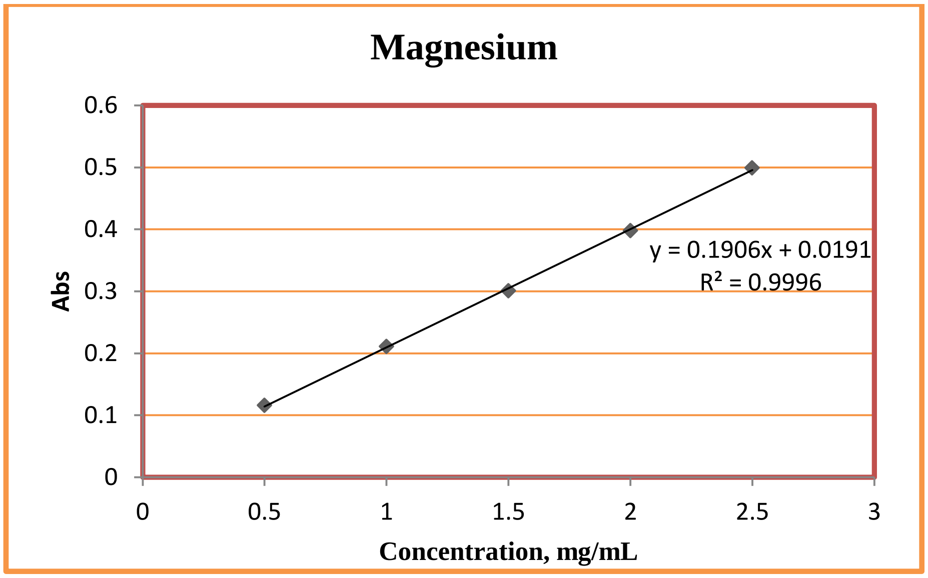
(Source: Ambo Agriculture station NMA, 2017)

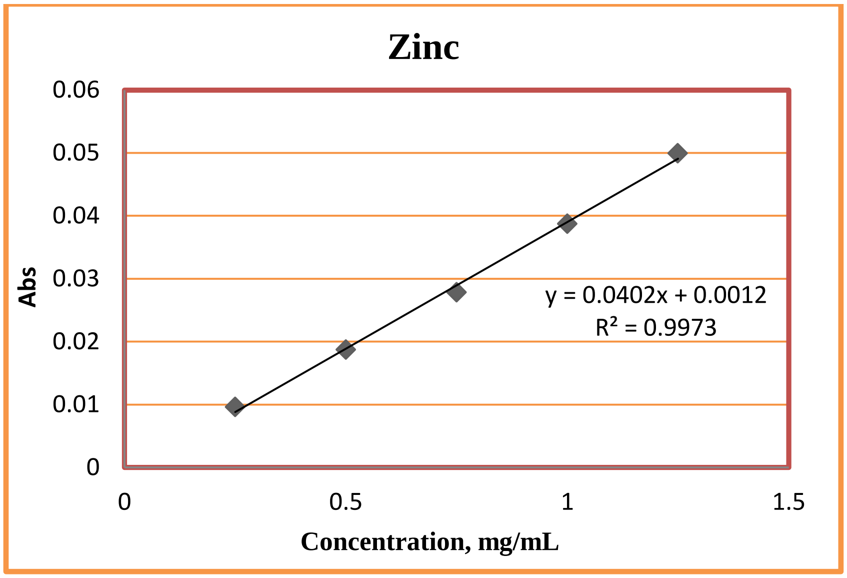
Annex 4- Regression equation Vs. Glucose concentration

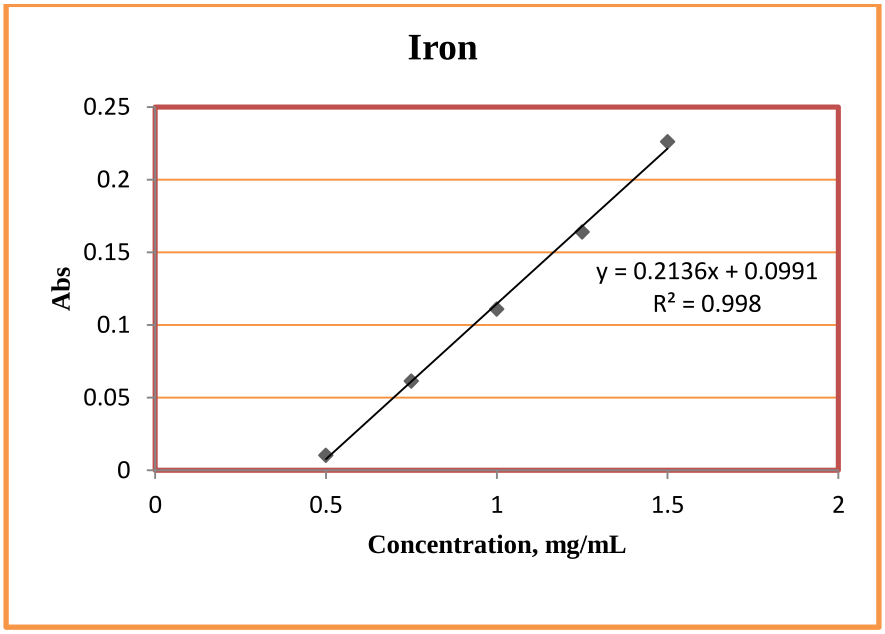


Annex 5- Calibration Curve for Minerals (Ca, Mg, Zn and Fe)






Zinc
Abs
Concentration, mg/mL
y = 0.0402x + 0.0012
R² = 0.9973
0.06
0.05
0.04
0.03
0.02
0.01
0
0
0.5
1
1.5


Iron
Abs
Concentration, mg/mL
y = 0.2136x + 0.0991
R² = 0.998
0.25
0.2
0.15
0.1
0.05
0
0
0.5
1
1.5
2