EFFECT OF SOIL AMENDMENTS ON THE QUALITY OF THREE COMMONLY CULTIVATED LETTUCE CULTIVARS

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MASTER OF PHILOSOPHY

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BY

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DECLARATION

I hereby declare that this thesis presented to the Department of Horticulture in partial fulfilment for the award of MPhil. Degree is a true account of my own work except for the references that have been duly acknowledged.

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DEDICATION

This research is dedicated to my mother Madam Renate Denutsui and late father Mr. Linus A. K. Amedor

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ABSTRACT

The effect of soil amended with poultry manure and NPK fertilizer on the quality of three cultivars of lettuce; Great Lake, Eden and Trinity cultivated in KNUST, were assessed and analysed. The cultivars were raised, harvested and stored at the Department of Horticulture of the Kwame Nkrumah University of Science and Technology, Kumasi. The harvested leaves were assessed for calcium, iron, vitamin C, chlorophyll, carotenoid level, weight loss and dry matter content as well as *E. coli* and faecal enterococci contamination. The treatments showed a significant effect ($p<0.05$) on the quality parameters except weight loss, dry matter content, *E. coli* and faecal enterococci load. The cultivars also varied ($p<0.05$) with regards to the quality indicators assessed. Similarly, the interaction of the treatments and cultivars showed significant differences. The results revealed the stream water used for irrigation as the lead source of the bacterial contaminants. The highest level of *E. coli* and faecal enterococci contamination ranged from 4.09 – 4.58 log cfu/g and 2.49 – 2.56 log cfu/g while the lowest loads recorded were 0.50 – 0.90 log cfu/g and 1.57 – 1.81 log cfu/g, respectively. Great Lakes harvested from plots amended with NPK was the only sample to have had no *E. coli* pathogens. Only few of the samples recorded contamination level below the satisfactory microbiological hygiene critical level of 2 log cfu/g.

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LIST OF ABBREVIATIONS

FAO	Food and Agricultural Organisation
FM	Farm Manure
FSANZ	Food Standards Australia New Zealand
HACCP-TQM	Hazard Analysis and Critical Control Points- Total Quality Management
HPA	Health Protection Agency
KNUST	Kwame Nkrumah University of Science and Technology
RCBD	Randomized Complete Block Design
WAT	Weeks After Transplanting
WBT	Weeks Before Transplanting
AAS	Atomic Absorption Spectrophotometer
pH	potential in Hydrogen
HCl	Hydrochloric Acid
AA	Ascorbic Acid
PCA	Plate Count Agar
MPN	Most Probable Number
NPK	Nitrogen, Phosphorus, Potassium
Ca	Calcium
Fe	Iron
Lsd	Least significant difference

CHAPTER ONE

1.0 INTRODUCTION

Lettuce (*Lactuca sativa L.*) is a native of Europe, Asia and Northern Africa and has been cultivated for over 5000 years. It grows best in a relatively cool season with monthly mean temperatures of 12.8 to 15.6 ^{0}C with an average minimum of 7.2 ^{0}C and an average maximum of 24 ^{0}C (Jeavons, 1995; Norman, 1997). Lettuce can be grown under protected cultivation in green house or in the open field (Mathew and Karikari, 1990; Filho, 2009). Nowadays, lettuce is grown all over the world in places of different climatic and soil conditions. Lettuce is a rich source of antioxidants, Vitamin A and C and phytochemicals which are anti-carcinogenic. It also provides some dietary fibre, carbohydrates, protein and a small amount of fat. Lettuce also provides calcium, iron and copper, with vitamins and minerals largely found in the leaf. Lettuce is usually consumed as a salad or shredded in a salad mix of onion, tomato, cheese and basil. In the market gardens of tropical regions including Ghana, early maturing iceberg type lettuce with three prominent cultivars (Eden, Trinity and Great lakes) are mostly cultivated. The productivity and quality of these lettuces depend on the growing conditions and soil amendments. Also, the difference in minerals and vitamins of the various cultivars of lettuce might also be due to the genotypic difference since they are grown under the same environment (Ojetayo *et al.*, 2011).

In developing countries, excessive amounts of inorganic fertilizers are applied to vegetables in order to achieve a higher yield (Stewart *et al*., 2005) and maximum value of growth (Badr and Fekry, 1998; Arisha and Bardisi, 1999; Dauda *et al.*, 2008). In Ghana commercial and subsistence farming has been and is still relying on the use of inorganic fertilizers for growing vegetable crops (Lampkin, 1990). This is because they are easy to use, quickly absorbed and utilized by crops. The widespread adoption of synthetic fertilizer and associated agricultural practices had a host of unintended consequences on our environment, the quality of our foods and health, and the sustainability of our food system. Organic manure can serve as alternative practice to inorganic fertilizers (Gupta *et al.*, 1988; Wong *et al*., 1999; Naeem *et al.*, 2006) for improving soil structure (Bin, 1983; Dauda *et al.*, 2008). Organic fertilizers can be used to reduce the amount of toxic substances such as nitrates produced by conventional fertilizers in vegetables like lettuce, hence, improving the quality of leafy vegetables as well as human health. Manure, considered a slow-releasing organic fertilizer provides small amounts of nutrients over an extended period and this makes it an acceptable form of mulch for plants (Hamilton, 2009). However, fresh manure is too strong for plants, as it contains excessive amounts of nitrogen, which can burn the plants. Some manure consists of urine as well, which is also high in nitrogen. The occurrence of high nitrate contents in vegetables cause a great concern among nutritionists. In the adult digestive tract, nitrates are reduced to nitrites under certain physiological conditions and present some hazards for human health. However, the release of available nitrogen from organic compounds during manure decomposition is very gradual. Poultry manure has long been recognized as the most desirable of these organic fertilizers because of its high nitrogen content and availability in most part of the country. It decomposes in the soil releasing nutrients for crop uptake. In a research conducted to find the effects of animal manure on different crops, it was reported that poultry manure was appreciably richer in plant nutrients than other animal manures (FAO, 2008).

Public concern about food quality and safety is steadily increasing. The judgment of fresh vegetables depends on visual characteristics as well as on nutritional quality. The concept of nutritional quality includes beneficial and harmful ingredients, taste, fragrance, freshness and shelf-life (Köpke, 2005) as well as the risk of toxic pathogens (Sagoo *et al*., 2001). Regarding lettuce, the marketable and nutritional quality depends heavily on the agronomic practices used during cultivation. Fast release of nitrogen (N) from fertilizers or a surplus of N can lead to an increase in nitrate content of plant tissues (Vogtmann *et al*., 1984; Sørensen *et al*., 1996), synthesis of N-containing compounds and a decrease in beneficial phytochemicals (Brandt *et al*., 2001). Contamination with enteric bacteria has been postulated for lettuce and other vegetables (Doyle, 1990).

The productivity and quality of vegetable crops, especially lettuce, therefore depends on the growing conditions and fertilizer application. Despite many investigations into the effect of fertilizers on growth and yield of lettuce, there is little information on their effects on postharvest quality of the crop such as; the nutritional components, bacterial contamination level and shelf-life. In view of this, there is the need for research to be conducted to determine the effect of soil amendments on the quality of three commonly cultivated lettuce cultivars.

The main objective of this study was to determine the effect of soil amendments on the quality of three commonly cultivated lettuce cultivars.

Specifically to;

I. Determine the effect of soil amendments on the nutritional composition of lettuce,

II. Determine the effect of soil amendments on bacterial contamination levels in lettuce with thermotolerant coliforms and enterococci and

III. Identify the effect of organic and inorganic fertilizers on the quality of lettuce after storage.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BACKGROUND INFORMATION

Lettuce (*Lactuca sativa*) is an annual plant of the aster or sunflower family *Asteraceae*. It is most often grown as a leaf vegetable and requires relatively low temperatures to prevent it from flowering quickly. Lettuce is most often used for salads, although it is also seen in other kinds of food, such as soups, sandwiches and wraps. Lettuce is a good source of vitamin A and potassium, as well as a minor source for several other vitamins and nutrients. There are 3 types of lettuce which are of economic importance to farmers in Ghana. These are; Crisp head also referred to as iceberg type. They are large, heavy, tightly folded, bristle or crisp textured with greenish wrapper leaves and whitish –yellow inner leaves. They are predominantly outdoor types but are now becoming more prominent in northern Europe. Cos or Romaine lettuce forms elongated leaves developing into large leaves shaped heads. Although this lettuce gives the appearance of toughness due to the heavy rib formation, the inner leaves are tender with good eating quality. Both crisp head and Romaine types may be classed as true shipping lettuce, while the Butter head are more for local shipping and are supplied mostly from nearby market garden. Butter head lettuce referred to as Bibb or Boston lettuce are bred to meet outdoor needs for summer conditions and green house requirements of the winter types. The United States and Northern Europe all share the same taste of this type of lettuce. It was noted that the butter head was having a higher resistance to mosaic disease and this type is an improved cultivar introduced by breeders (Nornecke, 1957).

Lettuce is a succulent leafy vegetable which has milky latex in the leaves and stems. The leaves vary in colour from light or yellow green to dark green. The flowers are composite, and yellow in colour. The stem which is cylindrical and contains latex vessels is about 10cm long in most cultivars but can reach 1m in length. The tap root has fibrous lateral branches and the leaves are almost sessile, arranged spirally in rosettes, variable in size from 10-25cm in length (Tindall, 1965).

2.1.1 Lettuce Production in Ghana and its related Problems

In Ghana, the urban population is growing at an estimated annual rate of 4.1% compared with the overall population growth of 3% (Ghana Statistical Service, 2002). The upsurge in urban population in recent times coupled with high rate of unemployment and other socioeconomic factors has led to increase in utilization of urban land and water resources for several purposes including food production particularly of high-value vegetables, which are needed to meet the urban diets and demand. This demand is not seasonal, necessitating year-round production, heavily dependent on irrigation (Sonou, 2001). Since the use of potable water for vegetable production is constrained, especially where over 40% of city dwellers in Ghana are without good drinking water, farmers often use low-quality water from different urban sources such as drains, shallow wells and streams. Consequently, municipal authorities and government ministries have raised concern regarding the potential health risks to consumers and wastewater irrigators. In Accra for example, the city health authorities have banned the production and sale of vegetables produced under such conditions (Armar-Klemesu *et al*., 1998). Continual productions on the same land have led to soil infertility necessitating the application of various soil amendments. Organic manure for instant help improves the soil fertility but when not allowed to fully decompose can be a source for contaminations.

2.2 ORGANIC AND INORGANIC FERTILIZERS

Plants, like other living things require energy for proper development and as such, sixteen essential elements are required for plant growth. Each is equally important to the plant; yet, each is required in vastly different amounts. Carbon (C), hydrogen (H), and oxygen (O) are derived from the atmosphere and soil water while, the remaining thirteen elements are supplied either from soil minerals and soil organic matter or by organic or inorganic fertilizers (Silva, 2000). A fertilizer as defined broadly by Nielsson (1968) is any material, organic or inorganic, natural or synthetic, that is placed on or incorporated into the soil to supply plants with one or more of the chemical elements necessary for normal growth. It is important to note that the quality of plant products can be considerably affected by plant nutrition. Moreover, the question is often asked whether there is any major difference in plant quality between plants supplied with organic or inorganic fertilizers. Mengel *et al*. (2001) reported that, in organic fertilizers such as farmyard manure, slurries and green manure have most plant nutrients which include potassium, magnesium and phosphate and are present in an inorganic form. Other nutrients, specifically, nitrogen and sulphur, are converted to inorganic forms by soil microorganisms before the absorption by plant roots takes place. Although plants may be supplied with organic fertilizers, they nevertheless take up inorganic nutrients derived from these organic materials (Mengel *et al*., 2001). Inorganic and organic fertilizers do, however, differ in the availability of the plant nutrients they contain. Nutrients in inorganic fertilizers are directly available to plant roots, whereas the nutrients of organic materials and especially organic nitrogen are of low availability. Chemical fertilizers are either sterile or have insignificant microbiological activity. They are primarily composed of water-soluble chemical salts and as such organic material rarely forms part of chemical fertilizers. Once these salts have been depleted from a chemical fertilizer, re-application is required in order to maintain the nutrient levels. With respect to moisture holding capacity and improvement of soil structure, chemical fertilizers have an insignificant effect, since they primarily consist of water-soluble salts. In the absence of sufficient detritus, soil organisms starve, humus content declines, and all the desirable properties of the soil decline as the top soil mineralizes. Nebel *et al*. (2001) also emphasizes the point that the case is not one in which chemical fertilizers do not have a role to play in enhancing crop production but rather, a keen understanding of the different roles played by organic materials and inorganic nutrients is required and that each type is used as necessary. This is important to consider since the exclusive use of organic material may provide insufficient amounts of one or more nutrients required to support plant growth.

In particular, the genetic structure of the soil bacterial community appeared to resist changes caused by addition of organic waste (Oldare *et al.*, 2011). Karanatsidis and Berova (2007) carried out research on pepper of “Buketen 50” and “Gorogled 6” cultivars, by using different organic and inorganic source of fertilizers, intended for production of red pepper for grinding. Pepper plants were grown in a phytostatic chamber under controlled conditions. The experiment was carried out with scheme as control – soil (no organic fertilizer application); Soil and organic – N fertilizer application. The indicated variants were formed during the pricking of the plants in phase 2-4 true leaf. Their results indicate that, applying organic -N fertilizer gave the vigorous plants expressed as plant height, leaves size as well as dry weight in both cultivars. The increase of dry biomass was mainly for the account of the increased mass of the above the ground organs. There was a positive effect of the fertilizer along with organic manure amendments upon the functional activity of the photosynthetic apparatus /increased content of photosynthetic pigments, improved leaf gas exchange. Michael *et al.* (2010) conducted a field experiment to evaluate the effects of farm manure (FM) and inorganic fertilizer application on the productivity of horticultural crops.

Two rates of FM selected (2 and 6 Mg ha-1 on dry weight basis) were combined with three rates of nitrogen (N) and phosphorus (P) fertilizer (0, 0), (61, 31) and (92, 46) kg ha^{-1} to six treatments. Four crops (onion, tomatoes, cabbage and potatoes, respectively) were planted in rotation on permanent plots. The treatments have a significant effect on biomass and yield of economic crops. In addition to the recommended inorganic fertilizer only 2 Mg ha^{-1} FM resulted in a significant increase in performance on the recommended rates. In addition, it was found that the reduction of fertilizer recommended by one third did not significantly reduce performance, if accompanied by 2 Mg ha^{-1} FM. It is not only to reduce the cost of production due to reduced use of fertilizer, but also improves soil quality leading to sustainability.

2.3 INFLUENCE OF ORGANIC AND INORGANIC FERTILIZERS ON QUALITY OF CROPS

The quality of vegetables cannot be defined in terms of any single, measurable characteristic. In fact, it is usually assessed by three critical criteria namely technological suitability (specific attributes which determine suitability for processing and storage), nutritional value (content of beneficial nutrients, such as protein, vitamins and content of harmful substances such as nitrates, natural toxins, pesticide residues and heavy metals) and appearance (size, shape, colour, freedom from blemishes and a taste specifically associated with individual products). In many cases, these quality characteristics can be measured quantitatively and thus provide a basis for comparison (Allemann and Young, 2002).

2.3.1 Internal Quality

2.3.1.1 Nutritional value

Regarding nutritional quality, more concerns are oriented towards negative aspects such as pesticide residues, food additives, fats, and to a lesser extent nitrates than towards positive factors such as protein, vitamins and trace elements. Organic produced vegetables have shown a decrease in the concentration of undesirable compounds such as Cd, Zn, Cu, NO_3^-, Pb and an increase in the concentration of desirable compounds such as vitamin A, B_1 (thiamin), B_2, B_{12} (cynocobalamin), C, E, β-carotene, soluble protein, carbohydrates, total sugars (sucrose, glucose and fructose) and mineral compounds (Ca, K, Mg, S and Na) compared to inorganic produced vegetables (Leclerc *et al.*, 1991; Nader *et al.*, 1993; Mozafar, 1994; Warman and Havard, 1996; Wong *et al.*, 1998; Premuzic *et al.*, 2002; Liu and Li, 2003; Rembialkowska, 2003; Suojala, 2003). Nader *et al.* (1993) and Rubeiz *et al.* (1993) reported that organic produced carrots and cabbage contained a high concentration of Cd and NO_3^- as well as a low level of β-carotene. It is well known that a high nitrate level is undesirable for human health because it is converted to nitrites which combine with haemoglobin and inhibits oxygen transport. Thus, to produce good quality vegetables it is important to grow them during the optimum time (spring) since nitrates accumulate in vegetables during cold climates (Liu and Li, 2003).

2.3.1.2 Calcium, Iron and Zinc content in Lettuce

A study by Magkos *et al*. (2003) evaluated the dry matter content of several vegetables and found that organically cultivated crops had higher dry matter content than those grown conventionally. These findings, however, are evident only for the plants that grow above the ground (leaf vegetables) such as spinach, lettuce, chard, savoy cabbage and white cabbage (Magkos *et al*., 2003). In all treatments, fertilization type resulted in significant effects in mineral composition of red lettuce on fresh mass basis. There was relatively higher zinc (Zn), iron (Fe) and calcium (Ca) contents in plants produced by bounce back compost. This can be attributed to the balanced quantity of nutrients in the bounce back compost. Magkos *et al*. (2003) reported that although a small number of studies have been published, slightly higher contents of minerals such as Fe, Ca, phosphorus (P), manganese (Mn), magnesium (Mg), zinc (Zn), copper (Cu), and potassium (K) have been obtained in organic vegetables; the majority of evidence, however, has revealed no significant differences between organic and conventional vegetables. Leaf mineral contents (Fe, Mn & Zn) were significantly affected by the application of organic and inorganic fertilizers (Ouda and Mahadeen, 2008). In general, each increase in organic manure and inorganic fertilizers dosages resulted in an increase in Fe, Mn and Zn leaf contents, but the differences in some cases were found non-significant. The highest broccoli leaf Fe (151 mg kg-1), Mn (223 mg kg-1) and Zn (5.02 mg kg-1) contents were observed by application the highest dosages of both organic manure (80 t ha-1) and inorganic fertilizer (60 kg ha-1), whereas the lowest leaf contents were observed by control treatment (Adediran *et al.,* 2004; Bokhtiar and Sakurai, 2005). The effect of organic manure on Fe-uptake could be due to the reason that organic carbon acts as a source of energy for soil microorganism, which upon mineralization releases organic acids that decreased soil pH and improves availability of makes Fe (Adediran *et al.*, 2004; Bokhtiar and Sakurai, 2005). Based on 100 g edible product of lettuce, fertilization had significant effects in calcium content of the lettuce. This can be attributed to relatively ample amounts of calcium in the chemical composition of chicken manure. Higher levels of manure had higher levels of calcium content as compared to lower levels. Similar results have been reported previously in broccoli (Ouda and Mahadeen, 2008) and in lettuce (Masarirambi *et al., 2010*).

2.3.1.3 Lutein, ß-carotene and polyphenol content in Lettuce

Rattler *et al.* (2006) reported that, fertilizer type and level also affected the lutein content. In both trials, the application of fresh and composted manure resulted in significantly lower lutein contents than with fertilizers that release nitrogen more rapidly. Increased fertilizer levels caused significantly higher lutein contents. Fertilizer type and level also affected the ß-carotene content. The spring trial showed an interaction between fertilizer type and level. Low levels of fresh and composted manure resulted in a significantly lower ß-carotene content than the same levels of nettle extract and mineral N-fertilizer. An increased level of composted manure showed a significantly increased ß-carotene content, while a higher level of fresh manure, nettle extract and mineral fertilizer showed no effect. In the summer trial, no interactions between fertilizer type and level were determined. Lettuce treated with fresh and composted manure had a significantly lower ß-carotene content compared with lettuce treated with nettle extract and mineral N-fertilizer. An increased level of all fertilizer types resulted in a significant increase in ß-carotene content (Rattler *et al.,* 2006). The polyphenol content was not affected by fertilizer type and amount in the spring trial. In the summer trial, fresh and composted manure application resulted in significantly lower levels compared with mineral N-fertilizer. A higher fertilizer level did not affect the polyphenol content for all fertilizer types. While there were no differences between the seasons with respect to lutein content, significant interactions between the experimental factors and the seasons were assessed for fertilizer and season for the parameters ß-carotene and polyphenol. The polyphenol content of lettuce fertilized with organic fertilizers was lower in plants grown in late summer than in crops grown in late spring. An application of mineral N-fertilizer in the spring trial resulted in a lower polyphenol content than in the summer trial (Rattler *et al.,* 2006). Similar to the polyphenols, the ß-carotene content of lettuce fertilized with organic fertilizers was lower in summer-grown plants than in spring-grown plants. The ß-carotene content of lettuce fertilized with mineral N-fertilizer was not affected by the season. These findings suggest an environmental influence on the ß-carotene and polyphenol formation in lettuce treated with organic fertilizers. Seasonal differences in ß-carotene and lutein levels between winter - and summer-grown kale cultivars were also reported by Mercadante and Rodriguez-Amaya (1991).

2.3.1.4 Chlorophyll content in lettuce

The estimate of the content of chlorophyll presented difference when compared the lettuce plants cultivated with treatments based on mixture of the conventional fertilization and manure. Leaf chlorophyll content was significantly higher when inorganic fertilizer adding with organic manure compared with using organic manure alone (Ouda and Mahadeen, 2008). Application of the highest dosages of organic manure (80 ton ha-1) with highest dose of inorganic fertilizer (60 kg ha-1) induced the highest leaf chlorophyll content, while the lowest chlorophyll content obtained by control treatment (Ouda and Mahadeen, 2008). These results agreed with the previous findings obtained on other vegetable crops (Arisha and Bradisi, 1999; Al-Tarawneh, 2005). A promotion effect of organic and inorganic fertilizers on chlorophyll contents might be attributed to the fact that N is a constituent of chlorophyll molecule. Moreover, nitrogen is the main constituent of all amino acids in proteins and lipids that acting as a structural compounds of the chloroplast (Badr and Fekry, 1998; Arisha and Bradisi, 1999).

2.3.1.5 Vitamin C

Schuphan (1974) found that spinach and lettuce grown organically were higher in ascorbic acid compared to those grown conventionally, using composted manure over organic fertilizers. Lairon *et al*. (1984) observed no difference in vitamin C content of kale and lettuce grown organically and conventionally. In addition, post-harvest factors which may introduce variables such as maturity at harvest could affect vitamin C content.

2.3.2 External Quality

2.3.2.1 Storage life

The application of organic fertilizer increases the firmness of cabbage heads as well as the shelf life of the produce. In a study conducted by Coleman (1993), lower storage losses were obtained in organic treated crops such as carrots, beetroot kohlrabi, potatoes and cabbage than inorganic treated vegetables. Similarly, Rembialkowska (2003) also reported a 22% mass loss in organic fertilized potatoes as compared to 30% of inorganic fertilized potatoes during storage.

2.3.2.2 Leaf dry weight/ dry matter

Takebe *et al*. (1995) reported that increments in leaf dry weight may be due to a combination of nitrogen with plant matter produced during photosynthesis such as glucose, ascorbic acid, amino acids and protein. Also, Tei *et al*. (2000) reported that increasing the rate of nitrogen fertilizer significantly increased the dry weight of leaves. In the first stages of growth there was a small increase in the rate of dry weight formation but by increasing plant growth and leaf area the amount of dry matter increased proportionally (Maryam and Ansari, 2007). Increasing the rate of nitrogen fertilizer affected leaf dry weight because nitrogen stimulates plant vegetative growth and increases leaf area; as a result increments in leaf area increase the rate of plant photosynthesis and thus high-weight will be obtained in the control treatment and the highest leaf fresh weight (798.69 g) at 120 kg N ha^{-1}.

The application of N fertilizer up to 120 kg N ha^{-1} increased the fresh weight of leaves significantly but as nitrogen fertilizer dose increased above 180 kg N ha^{-1} leaf fresh weight decreased. Between 60g N ha^{-1} and 180 kg N ha^{-1} no significant difference was found (Maryam and Ansari, 2007). The studies carried out by Rincon *et al*. (1998) on lettuce showed that increasing nitrogen fertilizer level to 100 kg N ha^{-1} increased its value. Tei *et al*. (2000) applied N fertilizer at different levels (0, 50, 100 and 200 kg N ha^{-1}) for two lettuce cultivars (cv. 'Audran' and cv. 'Canasta') and estimated N fertilizer rate to obtain maximum fresh weight at about 155 kg N ha^{-1} for both cultivars. Similarly, Broadley *et al*. (2000) and Tittonell *et al*. (2003) on lettuce (cv. 'Saladian R100' and cv. 'Grand Rapids', respectively) reported that fresh weight increased as N rate increased. Shoot fresh and dry weights of broccoli plants tended to increase by increasing dose of organic manure; however, this increment was not significant. Also, number of leaves per plant showed a little increase with increased organic manure dose (80 t ha-1). Each increase in inorganic fertilizer dose tended to increase number of leaves per plant compared with control. Generally, plots receiving a combination of organic and inorganic fertilizers produced slightly higher (P>0.05) fresh and dry weights of shoot. These results are in accordance with Shiralipour and Faber (1996) on broccoli; Wong *et al*. (1999) and Magnusson (2002) on Chinese cabbage and Abdelrazzag (2002) on onion. The highest numbers of leaves, fresh and dry weights of broccoli were obtained by application of 60 and 80 kg organic manure with 60 kg inorganic fertilizer. This variation might be due to the availability of nutrients especially nitrogen and could be due to the improvement of soil water holding capacity as mentioned earlier by Roe and Cornforth (2000).

Furthermore, organic manure activates many species of living organisms, which release phytohormones and may stimulate the plant growth and absorption of nutrients (Arisha *et al.*, 2003). Such organisms need nitrogen for multiplication. This is plausible reason that use of organic manure with inorganic fertilizer showed a beneficial effect on dry matter accumulation. Considerable variations in leaf dry matter of lettuce were observed amongst the treatments. Higher levels of chicken manure application had higher leaf dry matter while the one treated with synthetic fertilizer had the lowest. Magkos *et al. (2003)* established that vegetables cultivated on soils with higher amounts of organic fertilizers had higher dry matter as compared to those produced conventionally.

2.4 MICROORGANISMS ON LETTUCE

2.4.1 Escherichia coli

Escherichia coli is common in the normal microflora of the intestinal tracts of humans and other warm-blooded animals. Strains that cause diarrhoeal illness are categorized into groups on the basis of virulence properties, mechanisms of pathogenicity, clinical syndromes and antigenic characteristics. The major groups are designated as enterotoxigenic, enterohaemorrhagic, enteropathogenic, enteroinvasive, diffuse-adhering and enteroaggregative (Doyle *et al*., 1997). Fruits and vegetables can become contaminated with one or more of these groups while in the field or during post-harvest handling. Enterotoxigenic *E. coli* is a cause of traveler's diarrhoea, an illness sometimes experienced when individuals visit countries with food and water hygiene standards different from their own. Contaminated raw vegetables are thought to be a common cause of traveler's diarrhoea. Illness has been associated with consumption of salads (Merson *et al*., 1976; Mintz, 1994) and carrots (Centers for Disease Control and Prevention, 1994). Enterohaemorrhagic *E. coli* O157:H7 has more recently been recognized as a food-borne pathogen. Since cattle appear to be a natural reservoir for the pathogen, most outbreaks of illness have been associated with the consumption of contaminated, undercooked beef and dairy products. However, outbreaks have also been linked to lettuce (Ackers *et al*., 1996; Mermin *et al*., 1996), apple cider (Besser *et al*., 1993; Centres for Disease Control and Prevention, 1996; Steele *et al*., 1982), radish sprouts (Nathan, 1997) and alfalfa sprouts (Centers for Disease Control and Prevention, 1997). Enterohemorrhagic *E. coli* can grow on cantaloupe and watermelon cubes (Del Rosario and Beuchat, 1995), shredded lettuce (Diaz and Hotchkiss, 1996) and sliced cucumbers (Abdul-Raouf *et al*., 1993), and in apple cider (Zhao *et al*., 1993). Contamination of raw fruits and vegetables with enterohaemorrhagic *E. coli* O157:H7 may occur when cattle, and perhaps other ruminants such as deer, inadvertently enter fields, or when improperly composted cow manure has been applied as a fertilizer. The potential for contamination may be enhanced when fruits or vegetables have fallen from the plant to the ground and are then picked and placed into the handling and processing chain. Also, because contaminated manure may become airborne dust particles, it is possible that fruits on trees and vines may become contaminated. Workers on farms and in packing houses may also be a source of *E. coli* O157:H7. These mechanisms of contamination are somewhat speculative at present and must be thoroughly investigated before appropriate interventions can be introduced to reduce the risk.

2.4.2 *Enterococcus faecalis*

Enterococcus faecalis is a gram-positive bacterium that can cause a cultivar of nosocomial infections such as endocarditis, bacteremia, and wound infections of which urinary tract infections are the most common. These infections can be exceptionally difficult to treat because of drug resistance of many *E. faecalis* isolates.

2.4.3 Acceptable/permissible levels of microorganism in Lettuce

There are no worldwide acceptable levels of microorganism in vegetables but various departments of health concern have laid down some guidelines which can be considered safe for consumption. Some of these departments and their guidelines include;

- Australia New Zealand Food Standards Code(FSANZ, 2001)-

  This code made it clear that, food that contains uncooked fresh fruit and/or vegetables (eg salad) should have <3cfu/g to be considered good, 3 to <10^2 cfu/gto be considered acceptable, >10^2 cfu/g to be considered unsatisfactory for *E. coli* contamination. For *Staphlycoccusaureus* and *Bacillus cereus*<10^2 cfu/g is considered ‘good’, 10^2 to <10^3cfu/g is considered acceptable, 10^3 to

<10^4 cfu/g is considered unsatisfactory and greater than or equal to 10^4cfu/g is considered potentially hazardous.

- Hazard Analysis and Critical Control Points- Total Quality Management (HACCP-TQM) Technical Guide-lines lay down the microbial quality for raw foods. That is, <4cfu/g is rated good, 4-6.69 cfu/g is rated average, 6.69-7.69 cfu/g is rated poor and > 7.69 cfu/g is rated spoilt (Aycicek *et al*., 2006).
- Microbiological criterion in the HPA (PHLS) guidelines for the microbiological quality of ready-to-eat salad vegetables. *E. coli* is rated unsatisfactory when cfu/g is greater than 10^{2}. *Bacillus cereus* with 10^2-<10^4cfu/g is also rated unsatisfactory. *Staphylococcus aureus* with cfu/g greater than or equal to 10^4 is rated unacceptable (HPA, 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. EXPERIMENTAL SITE

The experiment was conducted at Department of Horticulture experimental field, Kwame Nkrumah University of Science and Technology.The area is located between latitude 6^{0} 40”26’ North and longitude 1^{0} 35” West and lies approximately 260m above sea level. The climate of the area is tropical maritime, which is characterised by a wet and dry season with a double maxima rainfall regime. The major rainfall season occurs between March and September, peaking in June and August. November to March is the main dry season making rainfall weakly bimodal. The mean annual rainfall is 1300mm and the mean temperature is 28 ^{0}C.The experiment was carried out in the dry season (October to December 2012).

3.2 SOIL AND MANURE ANALYSIS

Soil and manure analysis was performed before the commencement of field experiments and after cultivation. Total nitrogen was determined using Kjeldahl method. Available phosphorus was determined calorimetrically using Spectrophotometer. Potassium, calcium and Iron from soil, manure and plant tissues were determined by the use of Atomic Absorption Spectrophotometer (AAS). The soil pH was measured using pH meter. All tests were performed at Crop Science Laboratory-KNUST.

3.3 FERTILIZERS

Two types of fertilizers were used. They were organic and inorganic fertilizers. The organic fertilizer specifically used was decomposed poultry manure which was obtained from KNUST-Animal **S**cience Department and the inorganic fertilizer was N.P.K. 15-15-15. There was also a control plot with no treatment. The rate of 300 Kg/ha of inorganic fertilizer (N: P: K) equivalent to 0.054kg/1.80 m^2 was applied and incorporated into the soil 2 weeks after transplanting (WAT). The application rate of the poultry manure was 20 t/ha reported to 3.6 kg /plot of 1.80 m^2. This was incorporated into the soil 2 weeks before transplanting (WBT) to ensure microbial activity occurs.

3.4 LETTUCE CULTIVARS

The cultivars of lettuce used in this experiment were Eden, Trinity and Great Lakes all belonging to the Crisp/Iceberg family. Eden has bright green wavy leaves, big and dense head; Trinity has shining green leaves, early maturing and tolerant to bolting and tip burn whiles Great lakes produces large, crinkled leaves which form a dense head in the centre. These cultivars were chosen due to their prominence among most vegetable growers and consumers in the country. The seeds of the plant were purchased from a certified agrochemical shop in Kumasi. Lettuce plants were harvested after a normal growth period of 9 weeks.

3.5 EXPERIMENTAL DESIGN

The experimental design used was a 3×3 factorial in a Randomized Completely Block Design (RCBD) with three (3) replications. There was a total of 27 plots. Plot size was 1.5×1.2 m.Thus the experimental field was 48.60 m^2. The plots were separated with paths of 0.5 m, between replications and 0.5 m between treatments. The gross experimental area was 81.1 m^2 and 1m of borders was provided around the experimental field on which lettuce plants were grown.

3.6 PLANTING PATTERN

Seeds of lettuce were sown in a nursery and transplanted onto the field after three weeks. Healthy and vigorous seedlings were selected and transplanted to the field at a spacing of 30 cm×30 cm with a total of 20 plants per plot. Watering of the plants was done at regular intervals (morning and evening) with stream water and weeds were manually controlled by pulling.

3.7 LETTUCE SAMPLIING

The lettuce plants were harvested after a normal growth period of 9 weeks including the period in nursery. Six lettuce heads from each plot were pulled up with sterile disposable gloves, washed and were put into separate sterile polythene bags and transported on ice to the laboratory for nutritional and selected bacterial contaminants analysis.

3.8 DATA COLLECTION

3.8.1 WATER SAMPLING

Irrigation water sample used was stream water. Using a sterile bottle, water was filled leaving a headspace, cap replaced, tightened and sample then transported to the laboratory for bacterial analysis.

3.8.2 CHLOROPHYLL AND CAROTENOIDS

Chlorophylls and carotenoids were extracted using methanol 99.9 % as solvent. Samples were kept overnight in a dark cold room at 4 ^{0}C. Leaf pigments were immediately determined after extraction. Absorbance readings were taken at 665.2, 652.4 and 470 nm. Total chlorophyll and carotenoids levels were calculated by using Lichtenthaler's formula.

3.8.3 CALCIUM (Ca) DETERMINATION

Calcium was determined by ashing accurately weighed 1 g of dried and ground sample into glazed, high-form porcelain crucible for 2 h in a muffle furnace at 500 ^{0}C. The ashed sample was left to cool and 10 drops of deionised water followed by 3 – 4 ml of nitric acid were added to the sample. Excess nitric acid was evaporated by placing the sample on a hot plate set at 100 – 120 ^{0}C. The sample was returned to furnace and ashed for additional 1 h and after being cooled, the ash was dissolved in 10 ml hydrochloric acid and transferred quantitatively to 50 ml volumetric acid. In order to counteract chemical interferences, which have been fairly documented to depress calcium absorbance, a releasing agent in form of lanthanum (10000 µg/ml) was added in all replicates and standards to obviate combined interference effects.

3.8.4 IRON (Fe) DETERMINATION

Washed samples were fragmented and dried in an exhaust drier at 55 ^{0}C. The dried material was ground in a laboratory mill. From each sample, 2.5 g of dry plant matter was weighed out and mineralized in a muffle furnace at 450 ^{0}C. After complete mineralization, combusted samples were dissolved in 10% HCl and transferred to flasks of 50 cm^3 capacity. The concentration of iron was determined by the atomic flame absorption method using an AAS 3 Zeiss apparatus.

3.8.5 VITAMIN C (ASCORBIC ACID) DETERMINATION

Ascorbic acid (AA) was extracted by homogenizing 7 g of tissue in 6% metaphosphoric acid. Ascorbic acid content was determined by using a column Inertsil 3(5 µm, 4.6×250 mm) Rizzolo *et al*., (2002).

3.8.6 WEIGHT LOSS

This parameter was determined by observing the daily changes in weights of the sampled plants in the plastic bottles until their shelf-life were completed. This was carried out with the use of an electric sensitive balance.

3.8.7 DRY MATTER

Weight of oven-dry mass of edible leaves was taken after drying the harvested leaves at 65 ^{0}C for 48 hrs. The dry matter after shelf- life was also taken using the same procedure.

3.8.8 *E. coli* (THERMO-TOLERANT COLIFORMS)

From each of the positive tubes identified, a drop was transferred into a 5ml test tube of trypton broth and incubated at 44 ^{0}C for 24 hours. A drop of Kovac’s reagent was then added to the tube of trypton broth. All tubes showing a red ring colour development after gentle agitation denoted the presence of indole and recorded as presumptive for thermo-tolerant coliforms (*E. coli*). Counts per 100 ml were calculated from the Most Probable Number (MPN) tables.

3.8.9 FAECAL ENTEROCOCCI

Serial dilutions of 10^{-1} to 10^{-4} were prepared by picking 1ml of the sample into 9 ml sterile distilled water. One millilitre aliquots from each of the dilutions were inoculated on a Slanetz and Barltey Agar prepared on sterile Petri dishes. The Petri dishes were pre-incubated at a temperature of 37 ^{0}C for 4 hours to aid bacterial resuscitation. The plates were then incubated at 44 ^{0}C for a further 44 hours. After incubation all red, maroon and pink colonies that were smooth and convex are counted and recorded as faecal enterococci.

3.8.10 SHELF – LIFE DETERMINATION

Lettuce plants were placed in plastic bottles of about 10cm filled with 100 mls of water and stored in a room of 30 ^{0}C and atmospheric humidity of 71 %. The weights of samples in the plastic bottles and the appearance of the leaves were observed each day for leaf drooping, browning, and yellowing as these affects the products marketability quality. The produce shelf-life was determined by the number of days they were stored up to marketable or acceptable quality.

3.9 DATA ANALYSIS

Data collected were subjected to statistical analysis, using Statistix Analytical Software version 9. Means differences were separated at 5 % using Lsd.

CHAPTER FOUR

4.0 RESULTS

4.1 SOIL AND MANURE ANALYSIS FOR SOIL NUTRIENTS

The analysis for nutrient compositions in the soil and manure before planting as well as after harvest are shown in Tables 4.1 and 4.2, respectively. The result showed that, the soil was deficient in organic content, total nitrogen, calcium and potassium. It was slightly acidic while the manure, an alkaline. Soil analysis of the amended plots after the experiment showed that, plots amended with poultry manure performed the highest in all the parameters than the other amendments except with minimal level of total nitrogen due to the fact that, it was greatly used by the leafy vegetable for growth. This therefore suggests that poultry manure amended plots have greater chance of influencing the quality of the plant.

Table 4.1: Soil and manure analysis before experiment

Sample	Organic content (%)	Organic Matter (%)	Total N (%)	Ca^{2+} cmol/kg	K^{+} cmol/kg	Fe mg/kg	Available P mg/kg	pH
Soil	0.72	1.24	0.11	5.2	0.14	20	154	6.1
Manure	15.96	-	2.29	11.84	4.47	11	2.46	8.12

Table 4.2: Soil analysis after harvest

Sample	Organic content (%)	Organic Matter (%)	Total N (%)	Ca^{2+} cmol/kg	K^{+} cmol/kg	Fe mg/kg	Available P mg/kg	pH
Control	0.83	1.43	0.05	8.73	0.11	23.33	255.08	6.78
P.manure	0.89	1.53	0.07	8.93	0.16	25.33	330.20	6.78
N.P.K	0.83	1.44	0.07	8.80	0.15	30.00	238.84	6.67

Table 4.3: Shelf –life Data

Lettuce plants went bad after 4 days of storage in a room of 30 ^{0}C and atmospheric humidity of 71 %.

Sample	Number Of Days Stored
Lettuce Plants	4

Table 4.4: Bacterial contaminants detected in irrigation water sample used for lettuce cultivation.

Irrigation water	Bacterial contaminants	
	E. coli	Faecal enterococci
count (log cfu/g)	2.68	3.7

4.2 QUALITY PARAMETERS OF LETTUCE AFTER HARVEST

4.2.1:Effect of Soil Amendment on Quality Parameters of Lettuce after Harvest

4.2.1.1 Calcium

Lettuce grown on amended soils recorded equal and higher calcium content than the un-amended soil as can be seen in Table 4.5. They were significantly different ($p<0.05$).

4.2.1.2 Iron

The treatments showed a significant difference ($p<0.05$) in Iron content as shown in Table 4.5. Lettuce grown on poultry manure amended soil recorded the highest iron content (1.90 mg/g), followed by N.P.K.plots (1.84 mg/g) and the least by the control (1.77mg/g).

4.2.1.3 Vitamin C

A significant difference ($p<0.05$) in Vitamin C was recorded among the treatments as shown in Table 4.5. Lettuce grown on poultry manure amended soil (21.00 mg/g) had the highest level of vitamin C, followed by N.P.K. (18.26 mg/g) and the least, by the control (16.30 mg/g).

4.2.1.4 Carotenoid

A significant difference in carotenoid content was recorded among the treatments as shown in Table 4.5. Lettuce grown on poultry manure had significantly the highest carotenoid content (2.46 mg/g) followed by these on N.P.K.plots (2.28 mg/g) and the least on (2.08 mg/g) control plots.

4.2.1.5 Chlorophyll

N.P.K. (4.19 mg/g) and the control (4.17 mg/g) plots recorded the same chlorophyll content in the lettuce cultivated but were significantly lower than plants treated with poultry manure (4.24 mg/g) as can be seen in Table 4.5

4.2.1.6 Dry Matter

The soil amendments had no significant influence ($p>0.05$) on dry matter content of the lettuce as shown in Table 4.5.

4.2.1.7 Escherichia coli (E. coli)

The soil amendments did not significantly affect the concentration of E.coli on the lettuce as shown in Table 4.5.

4.2.1.8 Faecal Enterococci

Similarly, no significant difference was recorded among the treatments in terms of faecal enterococci contamination on the harvested lettuce (Table 4.5).

Table 4.5: Effect of Soil amendment on some quality parameters of lettuce after harvest

Treatment	Calcium (mg/g)	Iron (mg/g)	Vitamin C (mg/g)	Carotenoid (mg/g)	Chlorophyll (mg/g)	Dry matter (g)	*E. coli* *(log cfu/g)*	F. enterococci (log gfu/g)
P. Manure	43.56 a	1.90 a	21.00 a	2.46 a	4.24 a	5.00 a	2.61 a	2.21 a
N.P.K.	42.89 a	1.84 b	18.26 b	2.28 b	4.19 b	5.00 a	3.19 a	2.12 a
Control	41.63 b	1.77 c	16.30 c	2.08 c	4.17 b	5.02 a	3.06 a	2.14 a
Lsd (5%)	0.81	0.03	0.90	0.06	0.02	0.06	0.94	0.13
CV (%)	3.52	3.47	9.00	5.04	0.96	2.1	58.91	10.93

Figures followed with same alphabets are not significant.

4.2.2 Effect of cultivar on Quality Parameters of Lettuce after Harvest

4.2.2.1 Calcium

The cultivars recorded a significant difference ($p < 0.05$). The result in Table 4.6 shows that Great Lakes (43.41mg/g) is richer in calcium than Trinity (42.00 mg/g). Calcium level in Eden (42.67 mg/g) is significantly similar to Great Lakes and Trinity.

4.2.2.2 Iron

The level of iron in the cultivars was significantly different ($p<0.05$). In the increasing order, Great Lakes recorded the least, followed by Eden while Trinity had the highest iron content as shown in Table 4.6.

4.2.2.3 Vitamin C

Except for Trinity (18.26 mg/g) which significantly had the same level of vitamin C to the rest, Eden (19.15 mg/g) was richer and had the highest vitamin C than the Great Lakes (18.15 mg/g) as shown in Table 4.6.

4.2.2.4 Carotenoid

The cultivars generally had the same level of carotenoid after harvest and were significantly not different ($p>0.05$) as can be seen in Table 4.6.

4.2.2.5 Chlorophyll

A significant difference ($p<0.05$) levels of chlorophyll content were recorded among the cultivars as shown in Table 4.6. Except for Eden (4.20 mg/g) which statistically had the same chlorophyll content to the rest, Great Lakes (4.21mg/g) had higher chlorophyll compared to Trinity (4.19 mg/g).

4.2.2.6 Dry Matter

Dry matter content in the cultivars was significantly not different ($p > 0.05$) as can be seen in Table 4.6

4.2.2.7 Escherichia coli (E. coli)

E. coli contamination of Eden cultivar was significantly higher ($p < 0.05$) compared to the other lettuce cultivars as shown in Table 4.6.

4.2.2.8 Faecal Enterococci

Faecal enterococci contamination on Eden was significantly different ($p<0.05$) from Trinity and Great Lakes in decreasing order. They had 2.53, 2.10 and 1.85 log cfu/g faecal enterococci counts respectively as shown in Table 4.6.

Table 4.6: Effect of Cultivar on some quality parameters of lettuce after harvest

Cultivars	Calcium (mg/g)	Iron (mg/g)	VitaminC (mg/g)	Carotenoid (mg/g)	Chlorophyll (mg/g)	Dry matter (g)	*E. coli* *(log cfu/g)*	F. enterococci (log cfu/g)
Gt Lakes	43.41 a	1.79 c	18.15 b	2.25 a	4.21 a	5.01 a	2.05 b	1.85 c
Eden	42.67 ab	1.83 b	19.15 a	2.28 a	4.20 ab	5.01 a	4.22 a	2.53 a
Trinity	42.00 b	1.88 a	18.26 ab	2.29 a	4.19 b	5.00 a	2.59 b	2.10 b
Lsd (5%)	0.81	0.03	0.90	0.06	0.02	0.06	0.94	0.13
CV (%)	3.52	3.47	9.00	5.04	0.96	2.1	58.91	10.93

Figures followed with same alphabets are not significant.

4.2.3 Interaction Effect of Treatments and Cultivars on Quality Parameters of Lettuce after Harvest

4.2.3.1 Calcium

There was a significant interaction effect ($p<0.05$) of the treatments and cultivars on the calcium content of the lettuce as can be seen in Table 4.7. Great Lakes grown on soil amended with poultry manure recorded the highest calcium level (44.22 mg/g) and was significantly different from N.P.K.-Eden (42.78 mg/g), Control-Great Lakes (42.11mg/g), N.P.K.-Trinity (42.00 mg/g), Control-Eden (41.67 mg/g) and Trinity (41.11mg/g) in the decreasing order. These were statistically not different from each other. Likewise, Eden (43.56 mg/g) and Trinity (42.89 mg/g) cultivars grown on soil amended with poultry manure were significant not different and similar to the Great Lakes lettuce on the same soil.

4.2.3.2 Iron

The interactions showed a significant difference ($p<0.05$) on the iron content as can be seen in Table 4.7. Trinity (1.94 mg/g) and Eden (1.90 mg/g) grown on plots treated with poultry manure and Trinity (1.89 mg/g) cultivated on plots treated with N.P.K. had a similar iron content. They were significantly different from the rest except Great Lakes lettuce (1.84 mg/g) grown on plots treated with poultry manure. The three cultivars grown on the control plots and Great Lakes on N.P.K. treated plots were not different.

4.2.3.3 Vitamin C

Likewise, the interactions showed a significant difference ($p<0.05$) on the Vitamin C content of the harvested lettuce as shown in Table 4.7. Eden grown on plots treated with poultry manure recorded the highest vitamin C content (21.89mg/g), followed by Great Lakes(20.89 mg/g) and Trinity(20.22 mg/g) on poultry manure treated plots, Eden(19.11mg/g), Trinity(18.11mg/g) and Great Lakes(17.56 mg/g) on N.P.K. treated plots and the least, by Eden(16.44 mg/g), Trinity(16.44 mg/g) and Great Lakes(16.00 mg/g) on the control plots in the decreasing order.

4.2.3.4 Carotenoid

A significant interaction effect ($p<0.05$) was recorded between the treatments and cultivars as can be seen in Table 4.7. Trinity (2.49 mg/g), Eden (2.45 mg/g) and Great Lakes (2.44 mg/g) grown on poultry manure treated plots had a similar carotenoid level and were different from Trinity (2.34 mg/g), Eden (2.28 mg/g) and Great Lakes (2.23 mg/g) harvested from plots treated with N.P.K. as well as Eden (2.12 mg/g), Great Lakes (2.08 mg/g) and Trinity (2.04 mg/g) on the control plots in the decreasing order. The highest carotenoid level was recorded by the three lettuce cultivars grown on plots treated with poultry manure and the least, by the lettuce cultivars grown on control plots.

4.2.3.5 Chlorophyll

Trinity (4.56 mg/g) lettuce grown on plots treated with poultry manure contained the highest chlorophyll content and was significantly different ($p<0.05$) from the rest except Great Lakes (4.25 mg/g) grown on the same plots as can be seen in Table 4.7. Trinity (4.16 mg/g) harvested from the control plots had the least chlorophyll content and this was significantly not different from N.P.K.-Eden (4.18 mg/g), N.P.K.-Trinity (4.19 mg/g) and Great Lakes (4.19 mg/g) and Eden (4.18 mg/g) on the control plots.

4.2.3.6 Dry Matter

The interaction of the soil amendments and the cultivars showed a significant difference ($p<0.05$) on dry matter content as shown in Table 4.7. N.P.K.-Eden and Control-Trinity had 5.08g dry matter content which was the highest and different compared to the least, 4.91g recorded by Trinity grown on poultry manure treated plots. The highest was significantly not different compared to the rest except 4.97g, 4.94g and 4.91g in the decreasing order recorded by Control-Eden, N.P.K.-Great Lakes and poultry manure–Trinity respectively.

4.2.3.7 Escherichia coli (E. coli)

There was a significant interaction difference ($p<0.05$) between the soil amendments and the cultivars in terms of *E. coli* contamination as shown in Table 4.7. N.P.K.-Eden had suffered the highest *E. coli* contamination of 4.84 log cfu/g whiles N.P.K.-Great Lakes had no contamination of 0.00 log cfu/g. *E. coli* contamination on Trinity (1.49 log cfu/g and 1.54 log cfu/g) as a result of no and poultry manure amendment on the plots was significantly not different compared to the least. Also, Eden harvested from plots with no and poultry manure had statistically an equal level of contamination and were second to the highest.

4.2.3.8 Faecal Enterococci

The interaction showed a significant difference ($p<0.05$) among the means as can be seen in Table 4.7. Eden grown on plots with no (2.49 log cfu/g) and poultry manure (2.56 log cfu/g) treatments recorded the highest level of faecal enterococci. They were significantly different from Trinity (1.57 log cfu/g) and Great Lakes (1.81 log cfu/g) grown on poultry manure treated plots which had the least faecal enterococci contamination in the increasing order. The rest were similar but different when compared the highest and least.

Table 4.7: Interaction effect of Soil Amendment and Cultivars on some quality parameters of lettuce after harvest

Treatment*Cultivars		Calcium (mg/g)	Iron (mg/g)	VitaminC (mg/g)	Carotenoid (mg/g)	Chlorophyll (mg/g)	Dry matter (g)	*E. coli* *(log cfu/g)*	F. enterococci (log cfu/g)
P. Manure	Gt Lakes	44.22 a	1.84 bc	20.89 ab	2.44 ab	4.25 ab	5.06 ab	2.99 cde	1.81 c
P. Manure	Eden	43.56 ab	1.90 a	21.89 a	2.45 a	4.22 bc	5.01 abcd	3.31 abc	2.56 a
P. Manure	Trinity	42.89 abc	1.94 a	20.22 bc	2.49 a	4.56 a	4.91 d	1.54 def	2.26 b
N.P.K.	Gt Lakes	43.89 ab	1.79 cd	17.56 de	2.23 c	4.21 cd	4.94 cd	0.00 f	1.57 d
N.P.K.	Eden	42.78 bc	1.83 c	19.11 cd	2.28 c	4.18 de	5.08 a	4.84 a	2.53 a
N.P.K.	Trinity	42.00 cd	1.89 ab	18.11 d	2.34 bc	4.19 cde	4.99 abcd	4.73 ab	2.25 b
Control	Gt Lakes	42.11 cd	1.75 d	16.00 e	2.08 d	4.19 cde	5.02 abc	3.15 bcd	2.15 b
Control	Eden	41.67 cd	1.76 d	16.44 e	2.12 d	4.18 de	4.97 bcd	4.53 abc	2.49 a
Control	Trinity	41.11 d	1.79 cd	16.44 e	2.04 d	4.16 e	5.08 a	1.49 ef	1.79 cd
Lsd (5%)		1.41	0.06	1.57	0.11	0.04	0.10	1.63	0.22
CV (%)		3.52	3.47	9.00	5.04	0.96	2.10	58.91	10.93

Figures followed with same alphabets are not significant.

4.3 QUALITY PARAMETERS OF LETTUCE AFTER STORAGE

After 4 days of storage, all of the minerals, vitamin, pigmentation levels and weight suffered a significant loss except with the dry matter content. Likewise, there was a significant reduction in the level of microbial contamination.

4.3.1 Effect of Soil amendment on Quality Parameters of Lettuce after Storage

4.3.1.1 Calcium

Lettuce from plots treated with poultry manure and N.P.K. respectively were not different in calcium levels. They had the highest levels and were significantly different compared to the control as can be seen in Table 4.8.

4.3.1.2 Iron

The soil amendments acted differently on the iron content of lettuces at end of storage. In decreasing order, lettuce from poultry manure treated plots had the highest iron contents, followed by N.P.K. and the least, the control as can be seen Table 4.8.

4.3.1.3 Vitamin C

Similarly, different levels of vitamin C were retained by the lettuces after storage. From the highest to the least, lettuce from poultry manure treated plots came first, followed by N.P.K. and the control as can be seen in Table 4.8.

4.3.1.4 Carotenoid

Again, the soil amendments had different levels of carotenoid in the lettuce after storage. Lettuce from plots with poultry manure application had the highest carotenoid level, followed by N.P.K. and the least by the control as shown in Table 4.8.

4.3.1.5 Chlorophyll

Lettuce grown from poultry manure treated plots significantly had higher chlorophyll content at the end of storage. They were different ($p<0.05$) from lettuce from N.P.K. and control plots which were not different as can be seen in Table 4.8.

4.3.1.6 Dry Matter

The soil amendments showed no significant difference ($p>0.05$) at the end of storage as can be seen in Table 4.8.

4.3.1.7 Weight Loss

Likewise, no significant difference ($p>0.05$) was recorded among the soil amendments at the end of storage as can be seen in Table 4.8.

4.3.1.8 Parameters on Microbial contamination

The soil amendments showed no significant difference ($p>0.05$) on *E. coli* and faecal enterococci at the end of storage as can be seen in Table 4.8.

Table 4.8: Effect of Soil amendments on some quality parameters of lettuce after storage

Treatment	Calcium (mg/g)	Iron (mg/g)	Chlorophyll (mg/g)	VitaminC (mg/g)	Carotenoid (mg/g)	Dry matter (g)	Weight Loss (g)	*E. coli* *(log cfu/g)*	F. enterococci (log cfu/g)
P. Manure	42.22 a	1.84 a	4.22 a	19.89 a	2.39 a	5.23 a	27.43 a	2.38 a	2.08 a
N.P.K.	41.67 a	1.79 b	4.16 b	17.15 b	2.26 b	5.21 a	28.77 a	3.09 a	2.08 a
Control	40.59 b	1.75 c	4.15 b	15.85 c	2.03 c	5.21 a	29.40 a	3.23 a	2.06 a
Lsd (5%)	0.89	0.03	0.02	0.91	0.06	0.13	3.92	0.96	0.15
CV (%)	3.96	2.6	0.87	9.51	4.89	4.45	25.31	60.73	13.36

Figures followed with same alphabets are not significant .

4.3.2 Effect of Cultivar on Quality Parameters of Lettuce after storage

4.3.2.1 Calcium

The cultivars showed a significant difference ($p<0.05$) in the level of calcium at the end of storage as shown in Table 4.9. Great Lakes retained significantly, the highest level of calcium (41.96 mg/g), followed by Eden (41.59 mg/g) and the least, by Trinity (40.93 mg/g). Eden was not different from the rest of cultivars.

4.3.2.2 Iron

At the end of storage, a significantly high iron level was recorded in Trinity (1.84 mg/g). It was different from Great Lakes (1.77 mg/g) and Eden (1.76 mg/g) that were not different from each other as can be seen in Table 4.9.

4.3.2.3 Vitamin C

Eden (18.30 mg/g) retained significantly, a higher vitamin C content at the end of storage compared to Trinity and Great Lakes that had recorded the same level of 17.30 mg/g as can be seen in Table 4.9.

4.3.2.4 Carotenoid and Chlorophyll

No significant difference ($p>0.05$) was recorded among the cultivars of lettuce with regards to carotenoid and chlorophyll at the end of storage as can be seen in Table 4.9.

4.3.2.5 Dry Matter

Eden and Trinity cultivars at the end of storage had a higher dry matter content. Both were similar and different from Great Lakes which recorded a significantly low dry matter as shown in Table 4.9.

4.3.2.6 Weight Loss

No significant difference ($p>0.05$) was recorded among the cultivars at the end of storage as shown in Table 4.9.

4.3.2.7 Escherichia coli (E. coli)

The level of *E. coli* contamination in Great Lakes (3.58 log cfu/g) and Eden (3.06 log cfu/g) was significantly higher and different compared to Trinity (2.06 log cfu/g) as can be seen in Table 4.9.

4.3.2.8 Faecal Enterococci

Faecal enterococci contamination was significantly higher ($p<0.05$) in Eden compared to the rest of the cultivars that were not different from each other as can be seen in Table 4.9.

Table 4.9: Effect of Cultivar on some quality parameters of lettuce after storage

Cultivars	Calcium (mg/g)	Iron (mg/g)	Chlorophyll (mg/g)	Vitamin C (mg/g)	Carotenoid (mg/g)	Dry matter (g)	Weight Loss (g)	E. coli (log cfu/g)	F. enterococci (log cfu/g)
Gt Lakes	41.96 a	1.77 b	4.18 a	17.30 b	2.21 a	5.09 b	26.26 a	3.58 a	1.81 b
Eden	41.59 ab	1.76 b	4.17 a	18.30 a	2.23 a	5.30 a	29.79 a	3.06 a	2.46 a
Trinity	40.93 b	1.84 a	4.18 a	17.30 b	2.26 a	5.27 a	29.54 a	2.06 b	1.96 b
Lsd (5%)	0.89	0.03	0.02	0.91	0.06	0.13	3.92	0.96	0.15
CV (%)	3.96	2.6	0.87	9.51	4.89	4.45	25.31	60.73	13.36

Figures followed with same alphabets are not significant .

4.3.3 Interaction Effect of Soil Amendment and Cultivars on Quality Parameters of Lettuce after Storage

4.3.3.1 Calcium

Eden cultivar grown on plots treated with poultry manure retained the highest level of calcium (42.67 mg/g). It was significantly different ($p<0.05$) from Trinity harvested from N.P.K. (40.89 mg/g) treated plots as well as Eden (40.44 mg/g) and Trinity (40.22 mg/g) from the control plots in the decreasing order. Except for these, the rest of the interactions were not significant when compared against one another as shown in Table 4. 10.

4.3.3.2 Iron

Trinity harvested from poultry manure treated plots at storage end retained and recorded the highest level of iron of 1.90 mg/g. It was significantly different ($p<0.05$) from all of the interactions except iron retained by the same cultivar grown on N.P.K. treated plots (1.86 mg/g). Eden harvested from control plots had the least iron of 1.71 mg/g. This was statistically not different Great Lakes from both N.P.K. treated (1.76 mg/g) and control (1.75 mg/g) plots as well as Eden from N.P.K. (1.76 mg/g) treated plots as shown in Table 4.10

4.3.3.3 Vitamin C

Again, Eden harvested from plots treated with poultry manure recorded the highest level of vitamin C (20.78 mg/g) at the end of storage. It was significantly different ($p<0.05$) from all except Great Lakes (19.89 mg/g) from the same plots. Great Lakes, Trinity and Eden grown on the control plots recorded the least vitamin C content of 15.44 mg/g, 16.00 mg/g and 16.11 mg/g in the increasing order. They were significantly equal to Trinity and Great Lakes grown of N.P.K. treated plots as shown inTable 4.10.

4.3.3.4 Carotenoid

Trinity grown on plots treated with poultry manure (2.48 mg/g) at the end of storage maintained a significantly high level of carotenoid against the rest of the interactions. It was significantly different from all of the interaction means. Eden (2.37 mg/g) subjected to the same treatment was second to the highest. It was also different from the rest except Great Lakes (2.34 mg/g) and Trinity (2.33 mg/g) subjected to poultry and N.P.K. treatments respectively on the field. Trinity cultivar grown on control plots had the least carotenoid content (1.96 mg/g). It was not different from Great Lakes (2.03 mg/g) grown on the same control plots as shown in Table 4.10.

4.3.3.5 Chlorophyll

Great Lakes and Trinity subjected to poultry manure treatment on the field recorded the highest chlorophyll content at the end of storage. They were significantly different from all of the cultivars subject to the N.P.K. treatment and the control that had the least, except Eden, the second highest subjected to the same treatment as shown in Table 4.10.

4.3.3.6 Dry Matter

Trinity subjected to poultry manure treatment on the field had the highest dry matter content (5.42 g) and was significantly different ($p<0.05$) from Great Lakes grown on plots subjected to N.P.K. (5.11g) and poultry manure (5.02 g) respectively in the decreasing order. The rest of the interaction means were significantly not different from one another as shown in Table 4.10.

4.3.3.7 Weight Loss

Trinity (33.17 g) and Eden (32.92 g) grown on the control and N.P.K. treated plots respectively suffered the highest weight loss while Great Lakes subjected to N.P.K. (25.84 g) and poultry manure (25.68 g) treatments suffered the least. Both categories were significantly different from each other. The rest of the interactions statistically recorded the same weight loss as shown in Table 4.10.

4.3.3.8 Escherichia coli

There was a significant difference ($p<0.05$) among the interaction means in terms of *E. coli* contamination as shown in Table 4.10. In order of increasing, poultry manure-Eden (0.50 log cfu/g) recorded the least of *E. coli* contamination, followed by N.P.K.-Trinity (0.94 log cfu/g), Control-Great Lakes (1.95 log cfu/g), poultry manure-Trinity (2.09 log cfu/g), Control-Trinity (3.15 log cfu/g) and the highest, by N.P.K.-Eden (4.09 log cfu/g), N.P.K.-Great Lakes (4.23 log cfu/g), poultry manure-Great Lakes and Control-Eden (4.58 log cfu/g).

4.3.3.9 Faecal Enterococci

Similarly, Eden subject to poultry manure and N.P.K. treatments on the field respectively recorded the highest faecal enterococci count as shown in Table 4.10. Both were significantly different ($p<0.05$) from N.P.K.-Trinity (2.20 log cfu/g), Control-Great Lakes (2.04 log cfu/g), Trinity (1.90 log cfu/g) and Great Lakes (1.83 log cfu/g) subjected to poultry manure treatment as well as Control-Trinity (1.77 log cfu/g) and the least, N.P.K.-Great Lakes (1.56 log cfu/g) except Eden grown on the control plots which had 2.40 log cfu/g contamination level.

Table 4.10: Interaction effect of Soil Amendment and Cultivars on some quality parameters of lettuce after storage

Treatment*Cultivars		Calcium (mg/g)	Iron (mg/g)	Chlorophyll (mg/g)	Vitamin C (mg/g)	Carotenoid (mg/g)	Dry matter (g)	WeightLoss (g)	*E. coli* *(log cfu/g)*	F. enterococci (log cfu/g)
P. Manure	Gt Lakes	42.33 abc	1.80 cd	4.21 a	19.89 ab	2.34 bc	5.02 d	25.68 b	4.56 a	1.83 de
P. Manure	Eden	42.67 a	1.83 bc	4.20 ab	20.78 a	2.37 b	5.26 abc	28.68 ab	0.50 c	2.50 a
P. Manure	Trinity	41.67 abcd	1.90 a	4.24 a	19.00 bc	2.48 a	5.42 a	27.92 ab	2.09 bc	1.90 de
N.P.K.	Gt Lakes	42.44 ab	1.76 de	4.17 bc	16.56 de	2.26 cd	5.11 cd	25.84 b	4.23 a	1.56 f
N.P.K.	Eden	41.67 abcd	1.76 de	4.15 c	18.00 cd	2.20 de	5.30 abc	32.92 a	4.09 a	2.49 a
N.P.K.	Trinity	40.89 cd	1.86 ab	4.16 c	16.89 de	2.33 bc	5.22 abcd	27.53 ab	0.94 c	2.20 bc
Control	Gt Lakes	41.11 bcd	1.75 ef	4.16 c	15.44 e	2.03 fg	5.14 bcd	27.27 ab	1.95 bc	2.04 cd
Control	Eden	40.44 d	1.71 f	4.16 c	16.11 e	2.11 ef	5.36 ab	27.77 ab	4.58 a	2.40 ab
Control	Trinity	40.22 d	1.78 de	4.14 c	16.00 e	1.96 g	5.16 bcd	33.17 a	3.15 ab	1.77 ef
Lsd (5%)		1.54	0.04	0.03	1.58	0.1	0.22	6.79	1.66	0.26
CV (%)		3.96	2.6	0.87	9.51	4.89	4.45	25.31	60.73	13.36

Figures followed with same alphabets are not significant.

CHAPTER FIVE

5.0 DISCUSSION

5.1 MINERALS AND VITAMIN COMPOSITION OF LETTUCE

Lettuce like other leafy vegetables is an important source of minerals and vitamins needed in our diets for good functionality and repairs of the human body. Fresh vegetables like lettuce is judged on visual characteristics as well as nutritional quality (Rattler *et al.*, 2006) such as minerals and vitamins composition and risk of toxic pathogens (Sagoo *et al.*, 2001). Results of the experiment showed a significant impact of the soil amendment, varietal difference on the level of minerals and vitamin composition of lettuces studied and analysed. The three lettuce cultivars, Great Lakes, Eden and Trinity were rich in Calcium, Vitamin C and had a considerable good amount of Iron in significantly different quantities. The difference in the minerals and vitamin level might also be due to the genotypic difference since they were grown under the same environment (Ojetayo *et al.,* 2011).

5.1.1 Calcium Composition of Lettuce

Calcium content of the fresh harvested lettuce cultivars were significantly different ($p<0.05$) of which Great Lakes recorded the highest, followed by Eden while Trinity had the least. Both the organic and inorganic fertilization showed a similar but high impact on the calcium content compared to the control and it is possible that Great Lakes which had the highest calcium content might have absorbed and stored significant high calcium from the growth medium. Also the interaction of the soil amendments and the cultivars further explains that, fresh Great Lakes with the highest calcium content was harvested from plots for treated with poultry manure (organic fertilizer).The high calcium content from the plot treated with poultry manure can be attributed to relatively ample amounts of calcium in the chemical composition of chicken manure (Adediran *et al.*, 2004; Bokhtiar and Sakurai, 2005).

There was a significant drop in the level of calcium in the lettuce after storage though the nutritional lost was insignificant. Regardless of the lost, Great Lakes cultivar had the highest calcium and the least, by Trinity. The results also show that Great Lakes performed well with either poultry manure or N.P.K. fertilization and even the control. The difference might also be due to the genotypic difference since they were grown under the same environment (Ojetayo *et al.*, 2011).

Also reduction in calcium content of lettuce after storage shows that vegetables deteriorate immediately after harvest irrespective of the storage method adopted (Babarinsa; 2000).Again,temperature and relative humidity are critical factors in the maintenance of quality during storage, which reconfirms the inconsistency of the mineral quality of lettuce irrespective of the fertilizer type used. This agrees with the findings of Babatola and Olaniyi (1997) and Olaniyi *et al*. (2010).

5.1.2 Iron Composition of Lettuce

Freshly harvested Trinity had the highest iron content followed by Eden and the least, in Great Lakes. This is completely a reverse trend with calcium content in lettuce. That is, cultivars of lettuce with significant higher calcium have considerable low iron content.

The poultry manure released a significant higher iron amount to the soil for lettuce uptake compared to N.P.K. and the least, by the control. The effect could be due to the fact that organic carbon acts as a source of energy for soil microorganism, which upon mineralization releases organic acids that decreased soil pH and improves availability of Fe (Adediran *et al.,* 2004; Bokhtiar and Sakurai, 2005)**.** The effect of Fe on the cultivars as seen in the interaction showed Trinity had the highest iron content along with Eden. This outcome might have had a significant impact on the level of iron in the three lettuce cultivars studied though the differences in variety might have had much influence.

The analysis after storage however showed a slight change in the iron content. Trinity retained significantly higher iron content compared to Eden and Great Lakes which had a significant equal iron content. The effect of fertilization with the poultry manure and N.P.K. remained unchanged. The marginal losses in iron content observed in the cultivars could mainly be due to external factors which triggers high water loss in the lettuce as well as the living produce utilizing reserved nutrients to survive. Also reduction in Fe content of lettuce after storage shows that vegetables deteriorate immediately after harvest irrespective of the storage method adopted (Babarinsa; 2000).

5.1.3 Vitamin C

Vitamin C is known to serve as antioxidant that offer protection against some form of cancer. This along with some other phytochemicals and antioxidants reduce the risk of cancer of the respiratory system and intestinal tract (Wolford and Banks, 2013). Vitamin C may vary in concentration depending on the cultivar type. The results of the study showed varying concentration of vitamin C in the cultivars. Eden was richer in ascorbic content, followed by Trinity and the least in Great Lakes.The significant difference in vitamin C obtained among the cultivars might be due to the genotypic difference since they were grown under the same environment (Ojetayo *et al.*, 2011).

The organic (poultry manure) and control acted differently from each other with poultry manure contributing a significantly higher amount of the vitamin, especially to Eden, while the control performing poorly. Schuphan (1974) found that spinach and lettuce grown organically using composted manure were higher in ascorbic acid compared to those grown conventionally.The three cultivars also performed poorly on the control soil as they had the least vitamin C content.

The storage period and light had a significant impact on the lettuce by causing the three cultivars to lose vitamin C though it is said that cut vegetables normally retain vitamin C content longer. Vitamin C, like any antioxidants, is susceptible to degradation when exposed to oxygen or light (Gil *et al.*, 2006) after harvest. These could be the reason why the lettuces; Great Lakes and Trinity different from Eden lost Vitamin C increasingly. According to Kader (2002) and Lee and Kader (2000), postharvest losses in nutritional quality, particularly vitamin C content, can be substantial and the losses are enhanced by physical damage, extended storage duration, high temperatures and low relative humidity.

5.2 PHYTOCHEMICALS

5.2.1 Carotenoid

The three lettuce cultivars showed no significant differences in the carotenoid level in terms of varietal effect. This could mean that they all have similar genetic traits responsible for carotenoids synthesis. This is contrary to carotenoid concentration in some wild accessions of lettuce due to genetic variation reported by Mou (2005). Soil nutrients made available to the vegetable from different sources, that is organic and inorganic, significantly had an impact on carotenoid level in the lettuce after harvest as well as their shelf life. This effect was seen in the interaction of the cultivars and soil amendment materials. Numerous factors such as maturation stage (Rodriquez-Amaya, 2000), difference in the plant cultivars (Olson, 1999), soil type, effect of agrochemicals and cultivation conditions (Amaya-Farfan, 1999) may have interfered with the carotenoid content (Cardoso *et al.*, 2009) after harvest. Great Lakes, Eden and Trinity when subjected to poultry manure application, recorded the highest level of carotenoids. This is similar to the outcome of Rattler *et al.* (2006) where they had increased β-carotene content in lettuce as a result of using and increasing compost manure (organic fertilizer). The lettuce cultivars responded well to the inorganic fertilizer (N.P.K.) producing carotenoid levels close but different to the organic source. Rattler *et al.* (2006) also had a lower level of carotenoids (β-carotene) with application of mineral fertilizer. It thus means that, different fertilizer type (organic and inorganic) had a significant impact on the level of carotenoids. The least carotenoids level was produced by lettuce when grown on the control soil as a result of limited nutrients. That is, lettuce grown on soil deficient in nutrients and the effectiveness of a cultivar to respond to nutrients from different nutrient sources may affect the content of carotenoids.

A lost in the carotenoid levels in three cultivars occurred and hence, caused a slight difference in the quantities remained in them after storage. The results showed that, Trinity had the capability of retaining, significantly, the highest carotenoid level with poultry manure fertilization, followed by Eden and Great Lakes with the same treatment. Meanwhile, Trinity without any fertilizer treatment performed poorly in retaining the lowest level of carotenoids. This outcome could mean that the type of fertilizer applied can affect the rate at which lettuce loses carotenoids in storage. It is also possible that genetic makeup may have an effect on carotenoids lost caused by damage through injuries, temperature and humidity variation at storage. Amaya-Farfan (1999), attributed carotenoids difference in plants to several factors but the most relevant to the study after storage is the presence of damage to plant structure, exposure to light and storage condition which might have been a responsible cause. According to Azevedo and Rodriguez-Amaya (2005), carotenoids content is affected by climate alterations, with exposure to sunlight and higher temperatures, increasing the biosynthesis of carotenoids but, at the same time, inducing photo-degradation, thus reducing their levels in plants. This phenomenon could have taken place before end of storage.

5.2.2 Chlorophyll Content

Chlorophyll is a plant pigment which appears green and gives the green colour of plants. In the three cultivars studied, the chlorophyll content varied with Great Lakes (4.21mg/g) recording the highest and the least by Trinity (4.19mg/g) when analysed after harvest. However, the chlorophyll content at the end of storage of the lettuce was the same which may be due to its decomposition.Webexhibits (2013) reported that, chlorophyll decomposed in presence of sunlight and hence plants replenish by producing more. This claim could therefore support this outcome as the three cultivars of lettuce maintained their chlorophyll level immediately after harvest and had a reduction due to the fact that, they were taken out of their growth medium and parent stalk. Also, storage period and adverse condition within the storage area might have affected the lettuce and aided in the decomposition of the chlorophyll.

The treatments had a significant effect on the chlorophyll content of the harvested lettuces. Lettuce that had poultry manure treatment as fertilizer recorded significantly higher chlorophyll content than those with N.P.K. and no treatment application. Kempraj (2012) reported that the presence of nitrogen in soil and chlorophyll in plants are directly related. He further stated that chlorophyll could be used as an indirect indicator of nitrogen levels in fertilizer management. Based on this claim, it is possible that the poultry manure released more nitrogen to the lettuce than N.P.K.

Trinity (4.56mg/g) cultivar responded well to the poultry manure fertilization by producing the highest chlorophyll content and was not significantly different from Great Lakes (4.25mg/g) when subjected to the same treatment. Trinity cultivar however performed poorly when subjected to N.P.K. and the control treatment to produce the lowest chlorophyll contents. This may be due to inadequate nutrients especially nitrogen to be made available to crops for absorption.

There was a significant drop in chlorophyll due to the interaction of the treatments and cultivars at the end of storage. Trinity together with Great Lakes retained the highest chlorophyll content different from the chlorophyll recorded by the other interactions except with Eden, subjected to poultry manure application. Also, all cultivars retained very low chlorophyll content when subjected to N.P.K. This may be as a result of the organic fertilizer (poultry manure) releasing enough nutrients to the various cultivars.

5.3 DRY MATTER CONTENT

The soil amendment materials and the cultivars showed no significant individual effect after harvest. This could mean that, the different cultivars showed no genetic variation in terms of dry matter and thus have similar moisture composition. Furthermore, the fertilizer type might have not contributed to the dry matter content of the lettuce and any variations may be due to other factors. The analysis of dry matter content after storage was similar to that recorded after harvest except that, Great Lakes cultivar recorded a significantly lower dry matter that differed from Eden and Trinity which were similar. It is possible that the formation of the crisp head by Great Lakes reduced the surface areas of the leaves and hence, interfered with rate of respiration which results in moisture loss.

The interaction between the cultivars and treatments had a significant impact on the dry matter of lettuce both after harvest and end of storage. Eden and Trinity subjected to NPK and no treatment respectively had the highest dry matter content after harvest whiles Trinity grown with poultry manure fertilization recorded the least dry matter. It is generally reported that, leafy vegetables as well as root vegetables and tubers have higher dry matter contents when organically grown (Woëse *et al.*, 1997; Bourn and Prescott, 2002; AFSSA, 2003). The study revealed that difference may occur in vegetables that have similar or same genetic traits if organically grown. That is, after storage, Trinity and Great Lakes fertilized with poultry manure, had the highest and lowest dry matter content, respectively. The result also shows that Great Lakes have the tendency to retain enough or probably have a high moisture content compared to Trinity. That is, moisture content and dry matter content may be inversely related.

5.4 WEIGHT LOSS

The three cultivars of lettuce recorded no significant difference in terms of weight loss. It could be attributed to the fact they have similarities in moisture (water) composition regardless of difference in other characteristics. Likewise, type of fertilizer as a treatment also did not singly caused any significant effect. The absorption of water from the soil to other parts of the crop depends on the roots and basically, the three cultivars might have the same rooting system.

Contrary to the above, the interaction between the cultivars and the soil treatment slightly had a significant impact on the weight loss of lettuce. Eden and Trinity had the highest weight loss when fertilized with N.P.K. and the control compared to Great Lakes fertilized with N.P.K. and poultry manure which had the least weight loss.

5.5 MICROBIAL CONTAMINATION

5.5.1 Escherichia coli (E. coli)

E. coli contamination caused by the soil treatments were not significantly different. The level of contamination caused by these fertilizers in comparison to the control (plots without any fertilizer application) was also not different. This could mean that fertilizer type or nutrient source had no effect on *E. coli* contamination but rather may be caused by previous bad farm management or activities carried on the field. Irrigation water tested for *E. coli* load had 2.68 log cfu/g and hence deemed the source of contamination as well as inappropriate human handling during and after harvest.

Regardless of the above, Great Lakes (2.05) and Trinity (2.59) recorded the least contamination count of *E. coli* and were significantly different from Eden (4.22), which recorded the highest in log cfu/g. The, loose-broad and succulent leaves of Eden easily got contaminated from the soil toxic pathogens during irrigation as a result of water splash. Amoah *et al.* (2005) reported a similar finding that, general morphology of lettuce expose much of its surface area to the irrigation water and soil particles from the splashes and may have accounted for the high contamination.

Great Lakes with NPK treatment had no detection of *E. coli* but Eden recorded the highest *E. coli* count (4.84 log cfu/g) when subjected to the same treatment. The firm and compact heads for Great Lakes suggest a slight resistivity. After storage, Eden (0.50 log cfu/g) cultivars fertilized with poultry manure recorded the least count of *E. coli*. This may have happened as result of the soft and loosened leaves of Eden which are highly prone to water (weight) loss and hence, limit the growth of pathogens.

However, Trinity (2.06 log cfu/g) in general had a high reduction in *E. coli* count lower and different from the others. Great Lakes (3.58 log cfu/g) on the other hand, had an increased in *E. coil* but was not different from Eden (3.06 log cfu/g), which had a reduction. This result is different from the initial count of *E.* coli after harvest. The increased and high count of *E. coli* in Great Lakes could be as a result of the lettuce still making available, enough nutrients and food source for the multiplication and support of the toxic pathogens.

5.5.2 Faecal Enterococci

There were no significant difference in the level of faecal enterococci caused by the treatments. That is, the fertilizers type did not cause any changes in the level of the faecal contaminants on the lettuce and the source was from the irrigation water as it had 3.70 log cfu/g of the pathogens detected. A possible factor could also be from the soil. Level of the faecal contaminants, enterococci on the three cultivars, Great Lakes (1.81 log cfu/g), Trinity (2.10 log cfu/g) and Eden (2.53 log cfu/g) were highly different after harvest. The difference could be due to morphological variations of the cultivar to faecal enterococci. By comparison to other microbial contamination indicators, faecal enterococci count was the lowest. According to Obiri-Danso *et al.* (2001) and Beuchat (1998), enterococci are more sensitive to variations in environmental conditions and are easily knocked-off by sunlight and temperature. The interaction of the cultivars and the soil treatments also showed significant difference. Eden had the highest faecal enterococci count regardless of the type of fertilization. Thus, it is highly prone to faecal enterococci contamination. The result did not change much after storage. The load was still very high in Eden while Great Lakes and Trinity had a level count of faecal enterococci load.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1CONCLUSIONS

The soil treatment had a significant impact on the mineral composition, vitamin C content and chlorophyll concentration but not the carotenoids and the microbiological contaminations both after harvest and storage. The cultivars varied in the levels of Calcium, Iron, vitamin C and chlorophyll content due to variation in the expression of inherent genes with the cultivars and amount of nutrients made available to the lettuces. These minerals, vitamin C as well as the chlorophyll and carotenoids were relatively in high quantities. However, there was a significant drop in the mineral composition, chlorophyll, carotenoid and vitamin C. Similarly, the microbiological contaminants after storage, recorded a reduction in the *E. coli* and faecal enterococci pathogens.

The main source of contamination was deemed to be from the stream water as the soil treatments showed no significant impact on the level of microbial contaminants. Test for the level of contamination in the water showed that, the water was highly contaminated with *E. coli* and faecal enterococci with 2.68 log cfu/g and 3.70 log cfu/g respectively.

The highest level of E. coli contamination range from 3.31 – 4.84 log cfu/g after harvest and 4.09 – 4.58 log cfu/g after storage. The lowest *E. coli* counts were 1.49 – 1.54 and 0.50 – 0.90 log cfu/g after harvest and storage respectively. Also, the highest faecal enterococci counts were 2.49 – 2.56 after harvest and 2.40 – 2.50 log cfu/g after storage while the lowest counts were 1.57 – 1.81 log cfu/g and 1.56 – 1.77 log cfu/g after storage respectively.

Against all, Great Lakes treated with NPK showed zero count for *E. coli* after harvest but extremely high load of 4.23 log cfu/g after storage indicating that, human contact and activities like handling as well as storage place and condition could trigger high contamination level.

Only the lettuce with the lowest microbial counts were passed safe for eating (ready to eat) after harvest if compared to the satisfactory microbiological hygiene critical level of 2 log cfu/g set by European Commission (2005).

6.2 RECOMMENDATIONS

Based on the results of the experiment, highly decomposed and treated poultry manure could be encouraged for use as an alternative for other sources of fertilizers for the cultivation of lettuce as it records higher levels of minerals, vitamin C as well chlorophyll irrespective of the cultivar type.

Clean and treated water for irrigation of lettuce should be considered for cultivation of lettuce as the contaminated water raises microbial load.

Also, in homes of no refrigerators, freshly harvested lettuce plants should be kept in vessels of considerable amount of water as the process prolongs the produce shelf- life for four days.

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APPENDICES

Appendix 1: Analysis of Variance Table for Calcium after harvest

Source	DF	SS	MS	F	P
BLKS	2	41.210	20.6049		
S.Amendments	2	51.654	25.8272	11.47	0.0000
Cultivars	2	26.765	13.3827	5.94	0.0041
S.Amendments*Cultivars	4	1.975	0.4938	0.22	0.9269
Error	70	157.679	2.2526		
Total	80	279.284			

Grand Mean 42.691   CV 3.52

Appendix 2: Analysis of Variance Table for Chlorophyll after harvest

Source	DF	SS	MS	F	P
BLKS	2	0.01687	0.00843		
S.Amendments	2	0.07313	0.03656	22.25	0.0000
Cultivars	2	0.00735	0.00368	2.24	0.1144
S.Amendments*Cultivars	4	0.00778	0.00194	1.18	0.3260
Error	70	0.11504	0.00164		
Total	80	0.22017			

Grand Mean 4.2032   CV 0.96

Appendix 3: Analysis of Variance Table for Iron after harvest

Source	DF	SS	MS	F	P
BLKS	2	0.17951	0.08975		
S.Amendments	2	0.21862	0.10931	26.98	0.0000
Cultivars	2	0.09812	0.04906	12.11	0.0000
S.Amendments*Cultivars	4	0.01216	0.00304	0.75	0.5610
Error	70	0.28356	0.00405		
Total	80	0.79197			

Grand Mean 1.8342   CV 3.47

Appendix 4: Analysis of Variance Table for carotenoid after harvest

Source	DF	SS	MS	F	P
BLKS	2	0.29142	0.14571		
S.Amendments	2	1.96467	0.98233	74.84	0.0000
Cultivars	2	0.02900	0.01450	1.10	0.3371
S.Amendments*Cultivars	4	0.06289	0.01572	1.20	0.3195
Error	70	0.91884	0.01313		
Total	80	3.26682			

Grand Mean 2.2751   CV 5.04

Appendix 5: Analysis of Variance Table for vitamin C after harvest

Source	DF	SS	MS	F	P
BLKS	2	25.407	12.704		
S.Amendments	2	301.407	150.704	54.27	0.0000
Cultivars	2	16.222	8.111	2.92	0.0605
S.Amendments*Cultivars	4	8.815	2.204	0.79	0.5333
Error	70	194.370	2.777		
Total	80	546.222			

Grand Mean 18.519   CV 9.00

Appendix 6: Analysis of Variance Table for E. coli after harvest6

Source	DF	SS	MS	F	P
BLKS	2	3.393E+09	1.697E+09		
Cultivars	2	1.939E+10	9.695E+09	15.90	0.0000
S.Amendments	2	3.612E+09	1.806E+09	2.96	0.0582
Cultivars*S.Amendments	4	2.348E+10	5.869E+09	9.63	0.0000
Error	70	4.267E+10	6.096E+08		
Total	80	9.255E+10			

Grand Mean 34338   CV 71.91

Appendix 7: Analysis of Variance Table for Faecal enterococci after harvest

Source	DF	SS	MS	F	P
BLKS	2	21581	10791		
S.Amendments	2	48803	24401	3.10	0.0512
Cultivars	2	975448	487724	61.99	0.0000
S.Amendments*Cultivars	4	173695	43424	5.52	0.0006
Error	70	550701	7867		
Total	80	1770228			

Grand Mean 204.53   CV 43.37

Appendix 8: Analysis of Variance Table for dry Weight after harvest

Source	DF	SS	MS	F	P
BLKS	2	0.14741	0.07370		
S.Amendments	2	0.00963	0.00481	0.43	0.6498
Cultivars	2	0.00667	0.00333	0.30	0.7416
S.Amendments*Cultivars	4	0.21481	0.05370	4.84	0.0017
Error	70	0.77704	0.01110		
Total	80	1.15556			

Grand Mean 5.0074   CV 2.10

Appendix 9: Analysis of Variance Table for Calcium after shelf life

Source	DF	SS	MS	F	P
BLKS	2	21.210	10.6049		
S.Amendments	2	37.062	18.5309	6.88	0.0019
Cultivars	2	14.914	7.4568	2.77	0.0697
S.Amendments*Cultivars	4	4.494	1.1235	0.42	0.7958
Error	70	188.568	2.6938		
Total	80	266.247			

Grand Mean 41.494   CV 3.96

Appendix 10: Analysis of Variance Table for Chlorophyll after shelf life

Source	DF	SS	MS	F	P
BLKS	2	0.01891	0.00945		
S.Amendments	2	0.06898	0.03449	26.23	0.0000
Cultivars	2	0.00240	0.00120	0.91	0.4058
S.Amendments*Cultivars	4	0.00639	0.00160	1.21	0.3123
Error	70	0.09205	0.00131		
Total	80	0.18873			

Grand Mean 4.1769   CV 0.87

Appendix 11: Analysis of Variance Table for Iron after shelf life

Source	DF	SS	MS	F	P
BLKS	2	0.29939	0.14969		
S.Amendments	2	0.12145	0.06073	27.97	0.0000
Cultivars	2	0.11539	0.05769	26.57	0.0000
S.Amendments*Cultivars	4	0.01761	0.00440	2.03	0.1000
Error	70	0.15199	0.00217		
Total	80	0.70582			

Grand Mean 1.7919   CV 2.60

Appendix 12: Analysis of Variance Table for carotenoid after shelf life

Source	DF	SS	MS	F	P
BLKS	2	0.22593	0.11296		
S.Amendments	2	1.78674	0.89337	74.96	0.0000
Cultivars	2	0.03157	0.01579	1.32	0.2725
S.Amendments*Cultivars	4	0.22570	0.05643	4.73	0.0019
Error	70	0.83425	0.01192		
Total	80	3.10420			

Grand Mean 2.2302   CV 4.89

Appendix 13: Analysis of Variance Table for vitamin C after shelf life

Source	DF	SS	MS	F	P
BLKS	2	42.000	21.000		
S.Amendments	2	229.407	114.704	40.83	0.0000
Cultivars	2	18.000	9.000	3.20	0.0466
S.Amendments*Cultivars	4	8.815	2.204	0.78	0.5392
Error	70	196.667	2.810		
Total	80	494.889			

Grand Mean 17.630   CV 9.51

Appendix 14: Analysis of Variance Table for E. coli after shelf life

Source	DF	SS	MS	F	P
BLKS	2	5.894	2.9471		
S.Amendments	2	11.018	5.5090	1.78	0.1767
Cultivars	2	32.238	16.1192	5.20	0.0079
S.Amendments*Cultivars	4	136.486	34.1215	11.00	0.0000
Error	70	217.043	3.1006		
Total	80	402.680			

Grand Mean 2.8994   CV 60.73

Appendix 15: Analysis of Variance Table for Faecal enterococci after shelf life

Source	DF	SS	MS	F	P
BLKS	2	0.2650	0.13248		
S.Amendments	2	0.0032	0.00159	0.02	0.9796
Cultivars	2	6.2561	3.12803	40.73	0.0000
S.Amendments*Cultivars	4	1.9331	0.48328	6.29	0.0002
Error	70	5.3760	0.07680		
Total	80	13.8333			

Grand Mean 2.0759   CV 13.35

Appendix 16: Analysis of Variance Table for dry Weight after shelf life

Source	DF	SS	MS	F	P
BLKS	2	0.86247	0.43123		
S.Amendments	2	0.00691	0.00346	0.06	0.9381
Cultivars	2	0.68617	0.34309	6.35	0.0029
S.Amendments*Cultivars	4	0.45679	0.11420	2.11	0.0882
Error	70	3.78198	0.05403		
Total	80	5.79432			

Grand Mean 5.2210   CV 4.45

Appendix 17: Analysis of Variance Table for mean Weight loss

Source	DF	SS	MS	F	P
BLKS	2	1066.2	533.095		
S.Amendments	2	242.0	120.989	0.17	0.8451
Cultivars	2	930.5	465.230	0.65	0.5259
S.Amendments*Cultivars	4	3159.5	789.886	1.10	0.3630
Error	70	50218.1	717.401		
Total	80	55616.2			

Grand Mean 261.92   CV 10.23

Appendix 18: Pictures of lettuces on field and after storage

Plate 1: Lettuce at nursery and on plots after transplanting

Plate 2: Lettuce shelf life process

Appendix 19:Bacteria identification pictures

Plate 3: Escherichia coli formation