Potential of dairy digestate as a biofertilizer: Effects on growth, yield and phytochemicals of lettuce in hydroponics

By

Muhammad Faran

A thesis submitted to the School of Graduate Studies

In partial fulfillment of the requirements for the degree of

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Approved:

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Abstract

Lettuce (*Lactuca sativa* L) is a leafy vegetable containing an immense variety of minerals, vitamins and health-promoting secondary metabolites which are required for normal functioning of human body and health. Anaerobic digestate contains proportionately higher mineralized nutrients particularly ammonium-N (NH_4^+ -N) compared to undigested materials, and therefore has the potential to be used as a mineral nutrient source in soil based and soilless culture. A pilot study was conducted at St. Davids dairy farm site to investigate the effects of anaerobic dairy digestate (DD), inorganic nutrient solution (NS) and combine application of DD and NS on the growth, yield and quality indices of lettuce varieties in hydroponics under controlled environment conditions. Results showed that NS produced significantly higher leaf area (LA), chlorophyll content, root dry weight, yield, minerals and vitamins concentration of lettuce, compared to DD solution which produced lower LA, chlorophyll contents, yield and higher concentration of phenolics (chicoric acid, chlorogenic acid, luteoline, quercitin-3-b-d-gluconide, quercitin-3-glucoside and quercitin-b-malonyl) and antioxidants. Romaine lettuce showed superior performance and produced higher LA, chlorophyll contents, root dry weight, yield, minerals, vitamins, total phenolics, total antioxidants and polyphenols than Newham. It can be concluded that DD can be used as an organic fertilizer/nutrient source in hydroponics to enhance phenolics and antioxidants of lettuce without significant reduction in yield. Further research is needed to reduce Ammonium-N/Nitrate (NH_4^+/NO_3^-) in DD without dilution to avoid phytotoxic effects of NH_4^+ and loss of other macro and micro nutrient , and to establish the utilization of DD as a sole fertilizer source in hydroponic system.

General Summary

Lettuce, a common salad, contains various types of minerals, vitamins and health promoting phytochemicals required to maintain human health. However, growth, yield and quality of lettuce depends on environmental conditions, fertilization practices and potential of genotypes. Hydroponics is a soilless vegetable production system offers multiple benefits such as higher yield with increased number of crop cycles eventually leads to overall higher yield compared to traditional systems. Anerobic dairy digestat (DD) is rich in macro and micronutrients, particulalrly higher ammonium-N; required for plant growth and yield. It has been reported that DD application in soil enhanced growth and yield of lettuce; however, very little is known about its application in soilless culture or hydroponics. We used DD, nutrient solution (NS) alone and combined application of DD and NS in hydroponics to determine their effects on growth, yield, and quality of two lettuce varieties. Results showed that NS enhanced leaf area (LA), total chlorophyll content, root dry weight, yield, minerals and vitamin C in lettuce. Whereas, DD grown lettuce exhibited higher concentration of phenolics and antioxidants. On the other hand, Romaine variety showed superior agronomic performance, produced higher growth, yields and qualitative traits than Newham. On the basis of results, we conclude that DD could be used as an organic fertilizer and nutrient source for production of lettuce in hydroponics without compromising significant reduction in yield. Results further suggests that DD application enhanced phenolics and antioxidants in lettuce which enhance the immunity and other positive effects on human health. Further research is needed to reduce NH_4^+/NO_3^- in DD, without nutrient loss, to establish the utilization of DD as a sole fertilizer in hydroponic system. Using DD as nutrient source in hydroponics could a pragmatic approach to integrate food production and waste management to achieve food security and mitigating environmental effects.

Co-authorship statement

Manuscripts based on the chapter 2, entitled "*Effects of anaerobic dairy digestate on growth and yield of lettuce in a hydroponic greenhouse production system – a pilot scale study*" and Chapter 3, "*Influence of dairy digestate on phytochemical profile of hydroponically grown lettuce in controlled environment*" will be submitted to Scientia Horticulturae (Faran, M., Nadeem, M., Unc, A., Galagedara, L., Cheema, M. 2020) and (Faran, M., Nadeem, M., Manful, C., Unc, A., Galagedara, L., Cheema, M. 2020) respectively. Muhammad Faran, the thesis author will be the primary author and Dr. Cheema (supervisor), will be the corresponding and the last author. Dr. Adrian Unc (co-supervisor) and Dr. Galagedara (committee member) will be third and fourth authors, respectively. For the work in Chapter 2 and Chapter 3, Dr. Cheema and Dr. Unc wrote the research grants, Dr. Cheema developed the layout of this research trial and helped in results interpretation. Mr. Faran was responsible for the data collection, analysis, and writing of the manuscript. Dr. Charles Manful helped in lab analysis. Dr. Cheema, Dr. Galagedara, and Dr. Nadeem helped in statistical analyses and all authors helped in manuscript editing.

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Muhammad Faran

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List of Abbreviations

ABA – Abscisic acid

ANOVA – Analysis of variance

AsA – L-ascorbic acid

ASE – Accelerated solvent extractor

B – Boron

BERF – Boreal Ecosystem Research Facility

Ca – Calcium

Cu – Copper

DD – Dairy digestate

DW – Dry weight

EC – Electrical conductivity

Fe – Iron

$FeCl_3.6H_20$ – Ferric (III) chloride hexahydrate

FRAP – Ferric reducing ability of plasma

FW – Fresh weight

EDTA – Ethylenediaminetetraacetic acid

GH – Greenhouse

GMPase – GDP- mannose-pyrophosphorylase

HAA – Hydrophilic antioxidant content

HNO_3 – Nitric Acid

K – Potassium

KCl – Potassium chloride

LAA – Lipophilic antioxidant content

LA – Leaf area

LSD – Least Significant Difference

Mg – Magnesium

Mn – Manganese

MFS – Mineral feed solution

MPA – Metaphosphoric acid

N – Nitrogen

NS – Nutrient solution

NS+DD – Combination of inorgaic solution and dairy digestate

NH_4^+ – Ammonium

NH_3 – Ammonia

NO_3^- – Nitrate

NWD – New World Dairy Inc.

P – Phosphorus

PAL – Phenylalanine ammonia lyase

PO_4^{3} – Phosphate

QE – Quercetin equivalent

RH – Relative humidity

S – Sulfur

SE – Standard error

SO_4^{2-} – Sulphate

TAA – Total antioxidants

TCEP – tris(2-carboxyethyl)phosphine

TE – Trolox equivalents

TPC – Total phenolic contents

TPTZ – 2,4,6-tripyridyl-s-triazine

UHPLC – Ultra-high-performance liquid chromatography

V – Varieties

Zn – Zinc

Chapter 1

1. General introduction and literature review

1.1. Vegetables and health benefits

Availability, access, and use of adequate, safe and nutritious food is critical to maintain good health particularly for undernourished people. It is reported that, 49.5 million children under five years were affected due to malnutrition (FAO 2018). Poor nutrition causes physical and mental impairment in children, leads to loss of productivity. At least over a billion undernourished people live in Asia and sub-Saharan Africa whose diet is not only deficient in protein, carbohydrates, and vitamins but also in essential minerals as well (Mayer-Foulkes 2011). The amount and intake of quality food directly affects the people's nutritional status. The World Health Organization and the Food and Agriculture Organization of the United Nations recommend a minimum intake of at least 400 g of fruit and vegetables per person per day to achieve nutrition targets, but consumption far below this level is common in many countries. Consumption of cereals, meat, fish etc. can only provide specific minerals, proteins and vitamins but consumption of pulses and leafy vegetables will not only provide minerals, protein and vitamins but also health promoting bioactive compounds such as phemolic compounds (Kris-Etherton et al. 2002; Soetan et al. 2010).

Vegetables make a considerable part of the human diet in many parts of the world and add a significant amount of various nutrients, phytochemicals, vitamins and dietary fibers and hence have substantial contribution towards food security (Craig and Beck 1999; Wargovich 2000; Dias and Ryder 2011). Phytochemicals have strong antioxidants activities protecting against free radical damage through modifying metabolic activities and detoxification of carcinogens (Craig and Beck

1999; Southon 2000; Wargovich 2000; Herrera et al. 2009). Studies showed a positive correlation between the increased vegetable consumption and reduced risks of chronic diseases such as cardiovascular diseases, cancer and age-associated functional decrease (Hung et al. 2004; Morris et al. 2006; Pavia et al. 2006). These health-related beneficial effects are directly correlated with macro, micro-nutrients and bioactive compounds consumption that are present in the vegetables (Kris-Etherton et al. 2002; Soetan et al. 2010). Therefore, innovative production practices that could boost yield, minerals, vitamins and phytochemicals in vegetables are required.

1.2. Lettuce

Lettuce (*Lactuca sativa* L.) is one of the most popular leafy vegetable due to its ease of cultivation, low production cost and richness in nutrients (Correia 2013). It contains high amount of minerals (Squire et al. 1987), and is considered as a good source of various health promoting compounds and phytochemicals such as phenolics, vitamin C, folates, and carotenoids (Nicolle et al. 2004). Various minerals such as potassium (K), phosphorus (P), calcium (Ca), zinc (Zn), Iron (Fe), magnesium (Mg) and copper (Cu) are essential for better human health. Potassium intake is related to the lowering of blood pressure (Sacks et al. 1998), P is indispensable for the building of cell membranes (in phospholipids), intercellular energy metabolism, ATP and sugar generation (Roberts 2018), and Mg is vital for the activation of many enzymes related to replication of DNA and RNA synthesis and essential for muscle and nerve function (British Nutrition 2009). Ca is useful in blood clotting, boosting bone health and lowering the risk of osteoporosis (Gupta and Gupta 2014), Zn is vital for primary cellular function, improve immune system and antioxidant functions (Klouberta and Rink. 2015) and Iron has focused on its role in hemoglobin development and oxygen transport (Soetan et al. 2010). Cu is a part of numerous enzymes (Harris 2001) and is needed to produce red and white blood cells. It is not only helpful in maintaining the strength of the skin, blood vessels, epithelial and connective tissue throughout the body but also plays a role in the production of hemoglobin and keeps normal functioning of thyroid gland (Harris 2001). According to Medicine (2005) a 100 g of fresh lettuce provides 4–8% of recommended K intake, 0.3-3% for P and 3-9% of Mg, 15% Fe and 2-3% Zn of recommended for daily intake . According to Medicine (1997) 100 g of fresh lettuce make available 2–6% of the recommended Ca intake of 1000–1200 mg day^{-1} for adults.

Lettuce also contains ample amount of vitamins that are essential for metabolism (Survase et al. 2006). In addition to their various roles in physiological processes, vitamins play a critical role as food additives, health aids and medical therapeutic agents in humans. Vitamin C is common in lettuce (Kim et al. 2016), and is vital for normal metabolism, immune system and antioxidant functions (Carr and Vissers 2013), however, contents may vary in different varieties. For example, in Romaine green and red lettuce it ranges from 28-92 mg g^{-1} FW, and crisphead showed (28-48 mg g^{-1} FW) vitamin C contents (USDA 2015). Lettuce is a good source of folates, which act as cofactors in single-carbon transfer reactions (Scott et al. 2000). When folates are deficient, DNA synthesis decreases which affects the proper cellular functions (Shohag et al. 2012). According to the Medicine (1998), 400 mg day^{-1} folate intake is recommended for the adults, 100 g of lettuce can provide 18 % of this required folate. Pantothenic acid a water-soluble vitamin indispensable for the synthesis of coenzyme A (CoA) and acyl carrier protein (Miller and Rucker 2012), play a role in synthesis and degradation of fatty acid and a multitude of other catabolic and anabolic processes (Trumbo 2014).

Phenolic compounds refer to plant secondary metabolites responsible for plant defense systems (Dai and Mumper 2010; Lin et al. 2016). Phenolic compounds are abundant in all plant organs (Dai and Mumper 2010), more than 8000 polyphenols have been found in plants and involved in the sensory and nutritional properties of plants-based food (Karakaya 2004). For instance, total phenolic and total antioxidants vary from 21-73.9 mg g^{-1} sample FW and 15.19-127.09 mg $100g^{-1}$ FW among lettuce cultivars, respectively (Liu et al. 2007; Mampholo et al. 2016).

Phenolic compounds have shown beneficial effects against various human health issues such as inflammation, oxidative stresses, diabetes, age-related neurodegeneration, cancer and cardiovascular diseases (Cuevas-Rodri´guez et al. 2010; You et al. 2012). Phenolic compounds can be further divided into several groups depending on their chemical structure (Karakaya 2004). Phenolic acids and flavonoids are the subgroups reported in lettuce crops. For example, phenolic acids represent 70-94% of total phenolic content in green Romaine and crisp-head (iceberg) lettuce and 35-45% in the red leaf lettuce (Llorach et al. 2008). Common phenolic acids such as caffeic acid, chicoric acid, chlorogenic acid and their derivate (Zhao et al. 2007; Llorach et al. 2008). Chlorogenic acid (CA) is helpful in the reduction of renal oxidative stress and inflammation and exhibited an inhibiting effect on apoptosis and autophagy (Gagliardini et al. 2011). Chicoric acid has immuno-stimulatory properties, phagocytosis promoting activity (Bauer et al. 1989a). Besides, its antiviral activity (Pellati et al. 2004) has been reported to inhibit HIV integrase and replication (Healy et al. 2009a). Rajashekar et al. (2012) observed higher concentration of polyphenols in organically grown lettuce as compared to conventionally grown lettuce. They observed higher concentration of chicoric acid (0.2 - 1.1 mg g^{-1} FW), chlorogenic acid (0.15 – 0.5 mg g^{-1} FW) and quercetin 3-O-glucoside (0.03-0.10 mg g^{-1} FW) in organically grown lettuce, whereas, luteolin-7-O-glucoside (0.01 – 0.02 mg g^{-1} FW) was higher in conventionally grown lettuce.

However, growth, yield and quality of lettuce depends on application of organic or inorganic nutrients source, growth conditions and varieties. Inorganic fertilizers application is a common practice and seems very effective for crop production in commercial farming (Lampkin 1990; Masarirambi et al. 2010), due to its easy utilization, and quick absorption/uptake (Masarirambi et al. 2010). However, excessive use of inorganic N fertilizers may cause nitrate toxicity and cause human health issues such as gastrointestinal cancer (Hord et al. 2009b). Additionally, excessive use of inorganic N fertilizers application cause "luxuriant" growth, resulting in lodging (the bending over of the stems near ground level of grain crops, which makes it very difficult to harvest, and can dramatically reduce yield), enhance the risk of NO_3^- leaching and consequently increase cost of production, pollute water bodies and pose a serious threat to human health (Forge et al. 2016; Fan et al. 2017). On the other hand, organic fertilizers not only improve the soil organic carbon (SOC), total nitrogen contents, microbial biomass and microbial functional diversity which leads to increase soil productivity (Tamilselvi et al. 2015) but also diminish the toxic compounds in vegetables. For example, lettuce grown in organic fertilizers contains not only less amount of nitrate, but also higher concentration of phytochemicals compared to vegetables grown in inorganic fertilizers, which improves the quality of leafy vegetables and ultimately human health (Masarirambi et al. 2010). Yu et al. (2010) reported that application of concentrated biogas slurry in soil showed increased amino acids, protein, soluble sugar, β-carotene, tannins, and vitamin C in tomato fruits. Soil and specific environmental conditions play a significant role in crop production. Studies have shown that biogas slurry is a rich source of NH_4^+-N and other nutrients than their respective raw manure counterparts (Möller et al. 2008a). Consequently, environmental issues associated with land applications are potentially more prominent with biogas slurry such as surface and groundwater pollution and eutrophication of water bodies (Mulla et al. 2001). These concerns prompted the development of alternative methods to effective utilization of biogas slurry for crop production. One such alternative method could be utilizing biogas slurry or anaerobic dairy digestate (DD) in soilless culture or hydroponic system to mitigate environmental concerns while maximizing the nutrients use efficiency in DD.

Hydroponics refers to the method of growing plants in a water based, nutrient rich solution which does not use soil, instead the root system is supported using an inert medium such as perlite, rock wool, clay pellets, peat moss, or vermiculite. The basic philosophy is to allow the plants roots to come in direct contact with the nutrient solution, while also having access to oxygen, which is essential for plant growth. Additionally, there are many advantages of hydroponics such as easy control of nutrient composition, no soil contamination, short and fast crop growth cycle, high produce quality and good consumer acceptance, which make it an important plant production technique (Nicola et al. 2005; Petropoulos et al. 2016). In various regions, a lettuce crop growth cycle varies from 60-70 days in soil-based cultivation, whereas, it is 40-45 days in hydroponic cultivation (Cometti et al. 2013). Hydroponically grown plants contain more minerals as compared to the plants grown in the soil-based system (Coronel et al. 2008). Therefore, keeping in mind the environmental and land application issues, utilization of AD in soilless cultivation system could be a better method for lettuce production.

1.3. Anaerobic Digestate

The anaerobic digestion is also termed as biogas or bio-methanation process, was highlighted first time in 1776 by Alessandro Volta (Ahring 2003). Since then, it has been mainly utilized for biogas production from household waste and animal waste (Tani et al. 2006; Tambone et al. 2009). The popularity of bio-methanation process grew with significant interests around the globe mainly in Europe (Weiland 2010). For example, (a) treatment of the municipal sewage and sludge, (b) industrial wastewater treatment coming from agro-food and fermentation industries (c) waste from livestock, (d) organic waste treatment coming from the municipal solid waste (e) co-digestion of organic municipal solid waste and livestock waste (f) treatments of energy crop and (g) co-digestion of energy crops with animal slurries. These treatments and co-digestion yield biogas and anaerobic digestate (Ahring 2003; Gell et al. 2011). Anaerobic digestion is a widespread treatment that minimizes the negative environmental impact caused by the inappropriate management of agricultural wastes (Makádi et al. 2012). It also lessens the quantity of organic wastes by exploiting it for energy and heat production. Digestate quality as a fertilizer or amendment depends on in-gestate (material used for digestion) material and retention time. Longer retention time results in more effective methanogenesis (Szűcs et al. 2006). The thermophilic anaerobic digestate increases the rate of pathogenic bacteria elimination, number of fecal coliforms and enterococcus (Paavola and Rintala 2008).

1.3.1. Composition of digestate

Digestion process and composition of in-gestates determined the digestate quality, efficacy and use in agriculture. Mineral composition and organic matter are the key indicators of high quality digestate.

1.3.2. Nutrient composition of digestate

Digestate is a mixture of partially degraded organic matter, microbial biomass and various nutrients which varies among the digestate. Major macro-nutrient concentration of liquid digestate varies based on material and digestion type (Table 1).

Table 1: Variation in macro nutrients in the ingestate and digestion process

Type of ingestate	Type of digestion Process	Total Nitrogen (Nt)	NH_4^+-N	Total-P	Total-K	Reference
Swine manure	Mesophilic	2.93 (g L^{-1})	2.23 (g L^{-1})	0.93 (g L^{-1})	1.37 (g L^{-1})	(Loria et al. 2007)
Liquid cattle slurry	Mesophilic	4.27 (% DM)	52.9 (% Nt)	0.66 (% DM)	4.71 (% DM)	(Möller et al. 2008a)
Energy crops, cow manure slurry and agro-industrial waste	Thermophilic	105 (g kg^{-1} TS)	2.499 (g L^{-1})	10.92 (g kg^{-1} TS)	-	(Pognani et al. 2009)
Energy crops, cow manure slurry, agro-industrial waste and organic fraction of municipal solid wastes (OFMSW)	Thermophilic	110 (g kg^{-1} TS	2.427 (g L^{-1})	11.79 (g kg^{-1} TS)	-	(Pognani et al. 2009)
Cow manure, plant residues and offal	Mesophilic and Thermophilic	0.2013 (%m/m, fresh matter)	0.157 (%m/m, fresh matter)	274.5 mg kg^{-1} (fresh matter)	736.45 mg kg^{-1} (fresh matter)	(Makádi et al. 2008)
Clover/grass or pea straw or cereal straw or silage maize and clover/grass silage	Mesophilic	0.253 (%m/m, fresh matter)	0.176 (%m/m, fresh matter)	0.62 (% DM)	18.5 (% DM)	(Stinner et al. 2008)

Source: (Makádi et al. 2012)

Nitrogen (N) is known as a major plant nutrient and significant crop growth-limiting factor in agricultural production systems. It is also reported that the digestate contains a higher amount of N compared to composts because of carbon degradation to CO_2 and CH_4 and nitrogen preservation during anaerobic digestion (Tambone et al. 2009). Ammonium content in digestate reported was 60-80% of its total nitrogen (Makádi et al. 2012); however, Furukawa and Hasegawa (2006) reported 99% of NH_4^+-N from digestate obtained from the kitchen food and waste. It is reported that the concentration of NH_4^+-N is increased by the protein-rich feedstock such as dairy by-products and slaughterhouse waste (Menardo et al. 2011). Crops can immediately utilize the nitrogen when it is converted form organic-N to NH_4^+-N (Hobson and Wheatley 1992).

Digestate comprised of N, P and K in a sufficient amount (Tambone et al. 2010) which makes it more suitable for supplementing the macro-nutrients in the deficient soils. In addition to that Börjesson and Berglund (2007) reported available P and K in digestate makes it a useful material for supplementation in soil. The average ratio between P-K of digestate is about 1:3 which is suitable for grain and rapeseed (Makádi et al. 2012). Bachmann et al. (2016) and Insam et al. (2015) reported that application of digestate increased the plant available P and K to the same extend as highly soluble mineral P and K fertilizers and undigested slurries, which ultimately improves the nutrient reserves in soil and ultimately improves the plant nutrient availability. Various studies on digestate characterization showed that total P and total K concentration varies among the liquid digestate from 0.7–1.0 kg Mg^{-1} fresh matter (FM) and 3.5–5.2 kg Mg^{-1} FM respectively (Bauer et al. 2009; Möller et al. 2010) .

The value of anaerobic digestate as a fertilizer can be assessed directly from relative proportion of mineral nutrient essential to obtain same crop yield or universally recognized possibly inorganic fertilizers or organic fertilizer (Gong et al. 2011; Lafleur et al. 2012).

Anaerobic digestate obtained from animal slurries and maize ensilage co-digestion showed 30% higher nitrogen value than sole cattle and pig slurries. Various studies while using the anaerobic digestate for crop production revealed that the crops have similar or greater crop performances than undigested slurries and animal manures (Loria et al. 2007; Chantigny et al. 2007; Möller et al. 2008a; Bachmann et al. 2011). Whereas, some studies showed that anaerobic digestate was equally good as commercial fertilizers. For instance, digestate derived from household wastes showed quick releasing nitrogen comparable to synthetic fertilizer without any contamination when applied to spinach (*Spinacia oleracea* L.) and komastsuna (*Brassica rapa* var. perviridis L. H) (Furukawa and Hasegawa 2006). Likewise, liquid digestate obtained from the swine slurry was found as a good nutrient source as chemical fertilizers to spinach (*Ipomea aquatica* Forssk.) (Lam et al. 2002) and tomato (*Lycopersicon esculentum* Mill.) grown in greenhouse systems (Qi et al. 2005). A recent study on barley revealed that a liquid anaerobic digestate from household waste had the same results compared to NPK fertilizer (Knapp Haraldsen et al. 2011). It is also reported that a significant amount of mineral nitrogen could be substituted through biogas slurries in wheat crops (Tiwari et al. 2000). Similarly, the same results were found when liquid digestate obtained from the cattle manure was applied to the fields of timothy (*Phleum pratense* L.), legumes, sugar beet (*Beta vulgaris* L.) wheat (*Triticum aestivum* L.) and potato (*Solanum tuberosum* L.) (Hokkaido 2003). Chantigny et al. (2008) investigated the effect of swine liquid digestate on yield and nutrient uptake on maize. They reported that when incorporated as side-dressing, liquid digestate had a similar fertilizer value as mineral fertilizer have.

Micronutrient concentration varies among the digestate source and its origin. Alburquerque et al. (2012) assessed the fertilizer potential of digestate obtained from farm and agro industrial residues. They reported that the most abundant micronutrients in the digestate were Fe, Cu and Zn.

They further added that this high concentration in the digestate was due to use of pig and cattle slurry as a major co-digestion substrate. In another study conducted by Liu et al. (2011) investigated the biogas slurry in combination with Fe supplementation on lettuce growth and vitamin C concentration reported that Fe supplementation in digestate increased the vitamin C in lettuce. Tshikalange et al. (2020) investigated the comparative leaching capacity of biogas digestate used as soil amendment in comparison to inorganic fertilizer, consisting of three treatments such as cattle digestate, inorganic fertilizer and control for spinach production. Authors reported micronutrients concentration in the digestate used in the study containing (g kg^{-1}): Ca (6.1), Mg (1.77), Fe (3.41), S (2.5), Cu (0.12), Mn (2.15), Zn (0.54) and B (0.09). Similarly, Lind et al. (2020) used digestate consisting of crop residues, plant-based residues from food industry and 2% iron chloride. They observed 110 mg L^{-1} Ca, 23 mg L^{-1} Mg, 33 mg L^{-1} Fe , 27 mg L^{-1} S, 0.028 mg L^{-1}Cu, 1.2 mg L^{-1} Mn, 0.81 mg L^{-1} Zn and 0.21 mg L^{-1} of B respectively.

Organic matter (OM) is an integral part of digestate which refers to a large source of carbon-based compounds found within the environment. The OM content in the digestate reduced during the fermentation process of degradable carbon compounds (Stinner et al. 2008). For instance, Menardo et al. (2011) observed degradation of OM ranged between 11% and 38%. The digestate adequacy as an amendment relies on the OM content and most of the OM is converted into biogas, while remaining OM increase biological stability during the anaerobic digestion with the increase of more obstinate molecules such as lignin, humic acid, cutin, steroids and other complex complex organic molecules including proteins (Makádi et al. 2012). These aromatic and aliphatic molecules are potential precursors of humus with high stability (Tambone et al. 2009). It is reported that the concentration of these macromolecules increased during the anaerobic digestion (Pognani et al. 2009). It is also reported that the rate of lignin, cellulose and hemicellulose increased in OM after AD of cattle and pig dung (Kirchmann and Bernal 1997). The increase in lignin, cellulose and hemicellulose macromolecules in manures after anaerobic digestion were 2.4-26.8%, 13.9-14.2% and 7.3% respectively. The hemicellulose contents in pig dung decreased by 23.8% after anaerobic digestion, however, the increase in non-decomposable carbon contents of the AD is smaller than that of composts (Gómez et al. 2007). In contrast, higher decomposable carbon contents in digestate improve its effectiveness as a fertilizer which leads to increase crop roots proliferation and ultimately yield (Makádi et al. 2012). For example, Teglia et al. (2011) characterized the four digested products for their organic matter concentration. They reported that digestate obtained from organic fraction of municipal solid wastes contain 33.9% organic matter (), 16.5% from agricultural wastes, 15.3% in food processing wastes and 11.9% from wastewater treatment sludge.

1.4. Effects of anaerobic digestate or organic solution on growth and yield of lettuce

Various studies showed that application of inorganic fertilizer increased the growth and yield of the crop as compared to organic fertilizer application (Zandvakili et al. 2019). Increased crop growth and yield with inorganic fertilizer application is attributed to the availability in and uptake of nutrients from nutrient solution (Masarirambi et al. 2010). Wenke et al. (2009) evaluated application of digestate and inorganic nutrient solution on lettuce growth and yield. They reported that digestate application significantly enhanced yield and reduced the nitrate concentration in lettuce shoot compared to inorganic nutrient solution and therefore, digestate could be used as a biofertilizer to replace inorganic fertilizers solution in hydroponic systems. Another study conducted by Ronga et al. (2019) evaluated solid and liquid digestate in nine combination with agriperlite, standard solution, soil, peat moss and pelleted digestate for the production of baby leaf lettuce (*Lactuca sativa* L.) in hydroponic systems in three different experiments. They observed higher plant growth (root and shoot), yield and chlorophyll contents in digestate solution compared to other treatment combinations. Lind et al. (2020) investigated the effects of digestate and synthetic fertilizers on yield of bok choy (*Brassica rapa* var. chinensis) in hydroponic system and observed similar yields in biogas digestate and synthetic fertilizers application. They concluded that biogas digestate is a suitable plant nutrient source for hydroponic production of bok choy, considering productivity and circularity aspects. In India, liquid digestate obtained from cow manure, which is associated with on-farm product Panchavgaya (a mixture of five cow products i.e. dung, urine, milk, curd and ghee), had been incorporated into the organic-based farming system. It was observed that this Panchavgaya outperformed synthetic mineral fertilizer in corn and sunflower (*Helianthus annus* L.) crop production (Somasundaram et al. 2007). Ahmad and Jabeen (2009) investigated the effect of two organic fertilizers consisting of vermicompost and biogas slurry on sunflower, they reported the improvement in growth indices of sunflower in term of height, leaf area index and yield when fertilized with biogas slurry than vermicompost treatment. On the other hand, Liedl et al. (2006) observed less cucumber yield in anaerobic poultry litter digestate compared to inorganic commercial fertilizers in hydroponic system. However, digestate fed cucumber produced 7% higher grade one fruits compared to inorganic fertilizers. Barzee et al. (2019) evaluated the subsurface application of digestate biofertilizers on tomatoes production. They reported that the tomato plants grown in dairy manure digestate produced higher tomato yield compared to food waste digestate and mineral nitrogen fertilizer.

Different studies on characterization of digestate revealed that dairy digestate (DD) contains higher amount of NH_4^+-N/total N ratio (Möller et al. 2008a; Tambone et al. 2010). The organic N fraction in the excrements is further digested in the bioreactor where the retention times are much longer than in animal digestive tracts. This may explain the higher NH_4^+-N concentration generally observed in digested compared to undigested slurries. During the process of anaerobic digestion most of the organic nitrogen is converted into ammonium (Salminen et al. 2001; Liedl et al. 2006) which has a phytotoxic effect on plant growth (Tiquia et al. 1996). High concentration of NH_4^+-N triggers the changes in root architecture, reduced root growth, root/shoot ratio, and leaf chlorosis (Britto and Kronzucker 2002; Bittsánszky et al. 2015). Plant growth is most often limited due to availability of resources, particularly water and nutrients such as nitrogen (N) and phosphorus (P). Roots systems are vital and sole driver of water and nutrients uptake from the soil (Valverde-Barrantes et al. 2017) and nutrient solution. The root/shoot ratio is one measure to assess the overall health of the plant. Root elongation is the key phenotypic indicator of NH_4^+ toxicity in plants (Li et al. 2014). Britto and Kronzucker (2002) reported the stunted root growth in the presence of high NH_4^+ toxicity in root zone. Various studies showed that the NH_4^+-N toxicity greatly varies among the wild plant species and crops and accessions of Arabidopsis (Britto and Kronzucker 2002; Krupa 2003; Li and Shi. 2007). In another study Qin et al. (2008) observed root growth inhibition in Arabidopsis thaliana (mutant based root length assay). They observed that GDP- mannose-pyrophosphorylase (GMPase) enzyme synthesizes GDP-mannose which is essential for the biosynthesis of N-glycoprotein and L-ascorbic acid (AsA) (Conklin et al. 1999a). The GMPase activity in hsn1 and vtc1 mutants are NH_4^+-N sensitive (Qin et al. 2008) which triggers the glycosylation of defective protein in response to hypersensitivity in the roots (Qin et al. 2008; Barth et al. 2010). This defective protein N- glycosylation which is present in vtc1-1 contributes to cell wall and cell cycle defects, errors in protein folding and cell death in root cells which is directly associated with root growth inhibition mediated by the high NH_4^+ (Qin et al. 2008; Kempinski et al. 2011).

Chlorophyll content and LA are also important yield components contributing toward the final yield. Chlorophyll splays an important role in energy harvesting reactions during the photosynthesis process that help in CO_2 fixation in the presence of light and maximizing the synthesis of carbohydrates for energy production (Slamet et al. 2017) and subsequent plant growth and ultimately yield. Loqué et al. (2009) reported that the gene AMOS1/EGY1 (plastid metalloprotease), that contributes to chlorophyll content, depends on plastid signaling pathway, which is required for the expression of NH_4^+ responsive genes and the maintenance of chloroplast functionality, and is thus directly linked with plant photosynthesis process. Under NH_4^+ stress, the chloroplast receives the stress signal (the plasma membrane acting as the first site of perception of NH_4^+ stress), and triggering of AMOS1/EGY1-dependents retrograde signaling and recruiting downstream abscisic acid (ABA) signaling, to regulate the expression of NH_4^+ which possibly affects the plant growth and ultimately the yield (Li et al. 2012).

1.5. Effects of the anaerobic digestate or organic solution on quality of lettuce

Crop yield is considered an important economic parameter; however, quality of produce determines food safety and nutritious value. Digestate as an organic nutrient source considered to enhance crop growth, yield and quality of the crop (Makádi et al. 2012). Various studies reported that digestate increased the quality of vegetable crops. El-Shinawy and Gawish (2006) evaluated the effects of commercial organic and inorganic nutrient solutions on mineral composition of lettuce in soilless cultivation system during two growing seasons. The results indicated that lettuce grown in inorganic solution showed higher concentration of P, K, Ca and Mg, compared to organic solution which showed lower P, K, Ca and Mg, however, it exhibited higher total nitrogen. A study conducted by Zandvakili et al. (2019) reported that organically grown lettuce showed lower nutrient concentration and lower nitrate contents as compared to inorganic fertilized lettuce. They further observed higher quality of organically grown lettuce due to lower concentration of nitrate compared to chemically grown lettuce. Ibrahim et al. (2013) examined the effects of chicken dung (10% N:10% P_2O_5:10% K_2O) and inorganic fertilizer (15: 15: 15 NPK) with different N rates i.e. 0, 90, 180 and 270 kg N/ha on phenolic contents of Kacip Fatimah (*Labisia pumila* Benth). They observed higher concentration of total phenolics, flavonoids and ascorbic acid with chicken dung fertilizers than with inorganic fertilizer. Liedl et al. (2004) evaluated the effects of inorganic fertilizer and poultry liquid waste on lettuce production in hydroponic system and observed lower concentration of minerals in lettuce grown in poultry liquid waste except P, S and Zn were higher in poultry liquid waste compared to commercial inorganic fertilizer. Meagy et al. (2016) conducted the experiment to assess the effect of three mineral nutrient i.e. Hoagland solution, organic fertilizer and a chemical fertilizer (Jack's Professional, Peters Fertilizer Products Inc., 20-4.4-16.6 NPK), on nine cultivars of lettuce in a greenhouse production system. Authors observed 10% higher concentration of P, K, Ca, Mg and Zn in heritage cultivars than modern cultivars. Whereas, the mineral concentrations were significantly affected by nutrient regime; lettuce grown in inorganic fertilizers contained higher Ca, Mg, S, Zn, B and Fe concentration, whereas, P, K, Mn and Cu were higher in organic solution. Another study conducted by Liu et al. (2011) investigated the effect of biogas slurry in combination with different nutrient supplementation on vitamin C content of hydroponically grown lettuce. They reported that lettuce grown in biogas slurry in combination with ethylenediaminetetraacetic acid (EDTA-Fe; iron chelate) showed higher concentration of vitamin C as compared to other biogas treatments.

Phytochemicals such as phenolic acid which include various compounds have beneficial effects on human health. For example, chicoric acid has immune-stimulatory properties, promotes phagocytosis activity (Bauer et al. 1989a) and is also helpful to impede the HIV replication (Healy et al. 2009a). Similarly, chlorogenic acid is helpful against hepatitis B virus in human (Zuo et al. 2015). Quercetin and their derivatives, found in fruits and vegetables, have exclusive biological properties that may not only improve mental and physical health but also diminish the infection risk (Davis et al. 2009). Numerous studies described that polyphenols have anti-inflammatory and therapeutic properties that could reduce cardiovascular disease, neurodegenerative disorders, cancer, and obesity (Pérez-Jiménez et al. 2010). However, biosynthesis of polyphenols is highly dependent on fertilization practices and lower N fertilization (Olsen et al. 2008; Heimler et al. 2009). For example, in a recent study led by Mollavali et al. (2018) reported augmented concentration of several polyphenols such as quercetin-4′-O-β-D-glucoside and of quercetin-3,4′-di-O-β-D-glucoside in onion plants. This augmented polyphenol concentration is closely linked with increased phenylalanine ammonia lyase (PAL) enzyme activity in the plants grown in lower NO_3^- and predominantly higher NH_4^+-N solution. Higher NH_4^+-N in solution might have stimulated biosynthesis of phenolic compounds and increased expression of soluble peroxidase and superoxide dismutase enzyme in oxidative stress condition (Mihaljević et al. 2011).

Only very few studies have been conducted to investigate the effects of anaerobic DD on the growth and yield of vegetables as a sole fertilizer source (Lind et al. 2020). However, very little is known about the effects of DD alone and mix of DD and synthetic fertilizers on the growth, yield and phytochemical profile of lettuce in hydroponics, and therefore needs further investigation. Hence, we hypothesized that DD alone and mix of DD and inorganic nutrient solution will enhance the growth, yield, minerals, vitamins and phytochemical profile of hydroponically grown lettuce in controlled environment conditions.

Therefore, a pilot-scale study was initiated in a greenhouse in hydroponic settings with the following objectives.

1.6. Objectives

I. To evaluate the effects of DD, standard nutrient solution (NS) and their combined application (50% DD + 50% NS) on growth and yield of lettuce.

II. To determine the effect of DD, NS and combination of NS+DD on minerals and vitamins in lettuce.

III. To investigate the effects of DD, NS alone and combination of both nutrient solutions on bioactive compounds (phenolic content, flavonoids, total antioxidants) in lettuce.

1.7. References

Adams P (2002) Nutritional control in hydroponics. In: Savvas, D., Passam, H.C. (Eds.), Hydroponic Production of Vegetables and Ornamentals. Embryo Publications, Athens, Greece,

Ahmad R, Jabeen N (2009) Demonstration of growth improvement in sunflower (helianthus annuus l.) By the use of organic fertilizers under saline conditions

Ahring B (2003) Preface. In: Ahring BK (ed) Biomethanation II. Springer, Berlin.

Akhavan HR, Barzegar M (2017) Determination of water-soluble vitamins in 15 Iranian pomegranate cultivars and their variation after pasteurization and cold storage. . Int Food Res J 24:1429

Alburquerque JA, de la Fuente C, Ferrer-Costa A, et al (2012) Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass and bioenergy,. Elsevier 40:181–189

Arnon DI (1949) Copper enzymes in isolated chloropolasts. polyphenoloxidase in (Beta vulgaris). Plant Physiol 1–15

Bachmann S, Uptmoor R, Eichler-Löbermann B (2016) Phosphorus distribution and availability in untreated and mechanically separated biogas digestates. Sci Agric 73:9–17

Bachmann S, Wentzel S, Eichler-Löbermann B (2011) Codigested dairy slurry as a phosphorus and nitrogen source for Zea mays L. and Amaranthus cruentus L. J Plant Nutr Soil Sci 174:908–915

Barker A V., Eaton TE, Meagy MJ, et al (2017) Variation of mineral nutrient contents of modern and heirloom cultivars of cabbage in different regimes of soil fertility. J Plant Nutr 40:2432–2439

Barker A V., Maynard DN, Lachman WH (1967) Induction of tomato stem and leaf lesions, and potassium deficiency, by excessive ammonium nutrition. Soil Sci 103:319–327

Barth C, Gouzd ZA, Steele HP, Imperio RM (2010) A mutation in GDP-mannose pyrophosphorylase causes conditional hypersensitivity to ammonium, resulting in Arabidopsis root growth inhibition, altered ammonium metabolism, and hormone homeost. J Exp Bot 61:379-394.

Barzee TJ, Edalati A, El-Mashad H, et al (2019) Digestate biofertilizers support similar or higher tomato yields and quality than mineral fertilizer in a subsurface drip fertigation system. Front Sustain Food Syst 3:58

Bauer A, Mayr H, Hopfner-Sixt K, Amon T (2009) Detailed monitoring of two biogas plants and mechanical solid–liquid separation of fermentation residues. J Biotechnol 142:56–63

Bauer R, Reminger P, Jurcic K, Wagner H (1989a) Influence of Echinacea extracts on phagocytic activity. Z Phytother 10:43–48

Bauer R, Reminger P, Jurcic K, Wagner H (1989b) Influence of Echinacea extracts on phagocytic activity. Z Phytother 10:43–48

Benbrook C, Zhao X, Yáñez J, et al (2008) New Evidence Confirms the Nutritional Superiority of Plant-Based Organic Foods. State of Science Review; The Organic Center: Boulder, CO, USA,

Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal Biochem 239:70–76

Bittsánszky A, Pilinszky K, Gyulai G, Komives T (2015) Overcoming ammonium toxicity. Plant Sci 231:184–190

Bloch D, Monshausen G, Singer M, et al (2011) Nitrogen source interacts with ROP signalling in

root hair tip-growth. Plant, Cell Environ 34:76–88

Bonin CP, Potter I, Vanzin GF, Reiter W-D (1997) The MUR1 gene of Arabidopsis thaliana encodes an isoform of GDP-D-mannose-4, 6-dehydratase, catalyzing the first step in the de novo synthesis of GDP-L-fucose. Proceedings of the National Ac. Plant Biol 94:2085–2090

Boonpangrak S, Lalitmanat S, Suwanwong Y, et al (2016) Analysis of Ascorbic Acid and Isoascorbic Acid in Orange and Guava Fruit Juices Distributed in Thailand by LC-IT-MS/MS. Food Anal Methods 9:1616–1626

Börjesson P, Berglund M (2007) Environmental systems analysis of biogas systems—Part II: The environmental impact of replacing various reference systems. Biomass and Bioenergy 31:326–344

Bottoms TG, Smith RF, Cahn MD, Hartz TK (2012) Nitrogen requirements and N status determination of lettuce. HortScience 12:1768–1774

British Nutrition F (2009) Minerals and trace elements. Assessed on: March 06, 2020. Available at: https://www.nutrition.org.uk/nutritionscience/nutrients-food-and-ingredients/minerals-and-trace-elements.html?showall=1&limitstart

Britto DT, Kronzucker HJ (2002) NH4+ toxicity in higher plants: a critical review. J Plant Physiol 159:567–584

Bryson GM, Mills HA, Sasseville DN, et al (2014) Plant analysis handbook III. Athens, GA: Micro-Macro Publishing

Buchanan D, Omaye S (2013) Comparative Study of Ascorbic Acid and Tocopherol Concentrations in Hydroponic- and Soil-Grown Lettuces. Food Nutr Sci 10:1047–1053.

Cano A, Acosta M, Arnao MB (2003) Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (Lycopersicon esculentum Mill.). Postharvest Biol

Technol 28:59–65

Carr AC, Vissers MCM (2013) Synthetic or food-derived vitamin C—are they equally bioavailable? Nutrients 5:4284–4304

Chantigny M, Angers D, Belanger G, et al (2008) Yield and nutrient export of grain corn fertilized with raw and treated liquid swine manure. Agron J 1303–1309. https://doi.org/10.2134/agronj2007.0361

Chantigny MH, Angers DA, Rochette P, et al (2007) Gaseous Nitrogen Emissions and Forage Nitrogen Uptake on Soils Fertilized with Raw and Treated Swine Manure. J Environ Qual 36:1864–1872

Chapman HD, Pratt PF (1962) Methods of analysis for soils, plants and waters. Soil Sci 93:68

Cometti NN, Bremenkamp DM, Galon K, et al (2013) Cooling and concentration of nutrient solution in hydroponic lettuce crop. Hortic Bras 31:287–292

Conklin PL, Norris SR, Wheeler GL, et al (1999a) Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis. Proc Natl Acad Sci 96:4198–4203

Conklin PL, Norris SR, Wheeler GL, et al (1999b) Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis

Coronel G, Chang M, Rodríguez-Delfín A (2008) Nitrate reductase activity and chlorophyll content in lettuce plants grown hydroponically and organically. In International Symposium on Soilless Culture and Hydroponics 843. pp 137–144

Correia ECS (2013) Reação de cultivares de alface do grupo americano a Meloidoygine incognita, M. javanica e M. enterolobii (55f. Dissertação (Mestrado em Agronomia), Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, Brazil).

Craig W, Beck L (1999) Phytochemicals: Health Protective Effects. Can J Diet Pract Res 60:78–

84

Cuevas-Rodri´guez EO, Rodri´guez R, Dia VP, et al (2010) Inhibition of Pro-inflammatory Responses and Antioxidant Capacity of Mexican Blackberry (Rubus spp.) Extracts. J Agric Food Chem 58:9542–9548. https://doi.org/10.1021/jf102590p

Dai J, Mumper RJ (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15:7313–7352

Davis JM, Murphy EA, Carmichael MD (2009) Effects of the dietary flavonoid quercetin upon performance and health. Curr Sport Med Rep 8:206–213

Dias JS, Ryder E (2011) World Vegetable Industry: Production, Breeding, Trends. Hort Rev 38:299–356

Drennan MF, DiStefano TD (2010) Characterization of the curing process from high-solids anaerobic digestion. Bioresour Technol 2:537–544

Duarte TL, Lunec J (2005) When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C. Free Radic Res 39:671–686

Durazzo A, Azzini E, Lazzé MC, et al (2014) Antioxidants in Italian head lettuce (lactuca sativa var. capitatal.) Grown in organic and conventional systems under greenhouse conditions. J Food Biochem 38:56–61

El-Shinawy MZ, Gawish SM (2006) Effect of commercial organic nutrient solutions on growth and chemical composition of lettuce under agricultural soilless system. Egypt J Hortic 33:19

Environmental Protection Agency (1979) Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020

Errebhi M, Wilcox GE (1990) Plant species response to ammonium-nitrate concentration ratios. J Plant Nutr 13:1017–1029

Esteban R, Ariz I, Cruz C, Moran J (2016) Review: Mechanisms of ammonium toxicity and the quest for tolerance. Plant Sci 248:92–101.

Fan J, Xiao J, Liu D, et al (2017) Effect of application of dairy manure, effluent and inorganic fertilizer on nitrogen leaching in clayey fluvo-aquic soil: A lysimeter study. Sci. Total Env 592:206–214

FAO (2018) The state of food insecurity in the world 2018. Available at: http://www.fao.org/state-of-food-security-nutrition. Assessed on: (April 20, 2020). Public Library of Science

Fernández-Cirelli A, Arumí JL, Rivera D, Boochs PW (2009) Environmental effects of irrigation in arid and semi-arid regions. Chil J Agric Res 27–40

Fishman M, Friedman L (1989) Methods for determination of inorganic substances in water and fluvial sediments. US Department of the Interior, Geological Survey.

Forge T, Kenney E, Hashimoto N, et al (2016) Compost and poultry manure as preplant soil amendments for red raspberry: Comparative effects on root lesion nematodes, soil quality and risk of nitrate leaching. Agric Ecosyst Environ 223:48–58

Fullbloom (2020) Hydroponic Systems 101 | Learn The Basics of Hydroponics. Available at: https://www.fullbloomgreenhouse.com/hydroponic-systems-101/ (Assessed on July 05, 2020).

Furukawa Y, Hasegawa H (2006) Response of Spinach and Komatsuna to Biogas Effluent Made from Source-Separated Kitchen Garbage. J Environ Qual 35:1939–1947

Gagliardini E, Buelli S, Benigni A (2011) Endothelin in chronic proteinuric kidney disease. Contrib Nephrol 172:171-184.

Garbin ML, Dillenburg LR (2008) Effects of different nitrogen sources on growth, chlorophyll

concentration, nitrate reductase activity and carbon and nitrogen distribution in Araucaria angustifolia. Brazilian J Plant Physiol 20:295–303

Gavrilova V, Kajd M, Gjamovski V, Stefova M (2011) Separation, Characterization and Quantification of Phenolic Compounds in Blueberries and Red and Black Currants by HPLCÀDADÀESI-MS n. J Agric Food Chem 59:4009–4018

Gell K, van Groenigen J, Cayuela ML (2011) Residues of bioenergy production chains as soil amendments: immediate and temporal phytotoxicity. J Hazard Mater 186:2017–2025

Georgacakis D, Sievers DM, Iannotti EL (1982) Buffer stability in manure digesters. Agric Wastes 4:427–441

Gerendás J, Zhu Z, Bendixen R, et al (1997) Physiological and biochemical processes related to ammonium toxicity in higher plants. J Plant Nutr Soil Sci 160:239–251

Gliszczyńska-Świgło A (2007) Folates as antioxidants. Food Chem 101:1480–1483

Goddek S, Schmautz Z, Scott B, et al (2016) The effect of anaerobic and aerobic fish sludge supernatant on hydroponic lettuce. Agronomy 2:37

Gómez X, Cuetos MJ, García AI, Morán A (2007) An evaluation of stability by thermogravimetric analysis of digestate obtained from different biowastes. J Hazard Mater 149:97–105

Gong W, Yan X, Wang J, et al (2011) Long-term applications of chemical and organic fertilizers on plant-available nitrogen pools and nitrogen management index. Biol Fertil Soils 47:767–775

Gupta UC, Gupta SC (2014) Sources and deficiency diseases of mineral nutrients in human health and nutrition: a review. Pedosphere 24:13–38

Hao XJ, Hong JP, Gao WJ (2007) Effect of biogas slurry and biogas residues on quality of

greenhouse mini cucumber. [J] Soil Fertil Sci China 5:9

Hariadi YC, Nurhayati AY, Hariyani P (2016) Biophysical monitoring on the effect on different composition of goat and cow manure on the growth response of maize to support sustainability. Agric Agric Sci Procedia 118–127

Harris E (2001) Copper homeostasis: the role of cellular transporters. Nutr Rev 59:281–285

Hassan SA, Mijin S, Yusoff UK, et al (2012) Nitrate, ascorbic acid, mineral and antioxidant activities of Cosmos caudatus in response to organic and mineral-based fertilizer rates. Molecules 17:7843–7853

Haukioja E, Ossipov V, Koricheva J, et al (1998) Biosynthetic origin of carbon-based secondary compounds: cause of variable responses of woody plants to fertilization? Chemoecology 8:133–139

Healy EF, Sanders J, King PJ, Edwards Robinson WJ (2009a) A docking study of L-chicoric acid with HIV-1 integrase. J Mol Graph Model 27:584–589

Healy EF, Sanders J, King PJ, Robinson Jr WE (2009b) A docking study of L-chicoric acid with HIV-1 integrase. J Mol Graph Model 27:584–589

Heimler D, Isolani L, Vignolini P, Romani A (2009) Polyphenol content and antiradical activity of Cichorium intybus L. from biodynamic and conventional farming. Food Chem 114:765–770

Herrera E, Jiménez R, Aruoma OI, et al (2009) Aspects of Antioxidant Foods and Supplements in Health and Disease. Nutr Rev 67:140–144

Hjorth M, Christensen K V., Christensen ML, Sommer SG (2010) Solid—liquid separation of animal slurry in theory and practice. A review. Agron Sustain Dev 30:153–180

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil.

Circular. California agricultural experiment station, 347(2nd edit).

Hobson P, Wheatley A (1992) Anaerobic digestion – modern theory and practice. Elsevier Appl Sci 269

Hokkaido (2003) Research report of environment, resources, and recycling in Hokkaido, Sapporo. Civil Engineering Institute of Hokkaido, Sapporo (in Japanese).

Hoque MM, Ajwa HA, Smith R (2007) Nitrite and ammonium toxicity on lettuce grown under hydroponics. Commun Soil Sci Plant Anal 39:207–216

Hord NG, Tang Y, Bryan NS (2009a) Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 90:1-10.

Hord NG, Tang Y, Bryan NS (2009b) Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 90:1–10

Hung HC, Joshipura KJ, Jiang R, et al (2004) Fruit and vegetable intake and risk of major chronic disease. J Natl Cancer Inst 96:1577–1584

Ibrahim MH, Jaafar HZ, Karimi E, Ghasemzadeh A (2013) Impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kacip Fatimah (Labisia pumila Benth). Molecules 18:10973–10988

Insam H, Gómez-Brandón M, Ascher J (2015) Manure-based biogas fermentation residues– Friend or foe of soil fertility? Soil Biol Biochem 84:1–14

IPL (2020) Hydroponics Advantages And Disadvantages. Available at: https://www.ipl.org/essay/Hydroponics-Advantages-And-Disadvantages-PKNBD2NFCEDR (Assessed on July 05, 2020).

Joshi R, Adhikari S, Patro BS, et al (2001) Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. Free Radic Biol Med 30:1390–1399

Kapoulas N, Koukounaras A, Ilić ZS (2017) Nutritional quality of lettuce and onion as companion plants from organic and conventional production in north Greece. ElsevierScientia Hortic 219:310–318

Karakaya S (2004) Bioavailability of Phenolic Compounds. Crit Rev Food Sci Nutr 44:453–464

Kempinski CF, Haffar R, Barth C (2011) Toward the mechanism of NH4+ sensitivity mediated by Arabidopsis GDP-mannose pyrophosphorylase. Plant, Cell Environ 34:847–858

Kim MJ, Moon Y, Tou JC, et al (2016) Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). J Food Compos Anal 49:19–34

Kirchmann H, Bernal M (1997) Organic waste treatment and C stabilization efficiency. Soil Biol Biochem 29:1747–1753

Klouberta V, Rink. L (2015) Zinc as a micronutrient and its preventive role of oxidative damage in cells. Food Funct 6:3195–3204

Knapp Haraldsen T, Andersen U, Krogstad T, Sørheim R (2011) Liquid digestate from anaerobic treatment of source-separated household waste as fertilizer to barley. Waste Manag Res 29:1271–1276

Koester RP, Skoneczka JA, Cary TR, et al (2014) Historical gains in soybean (Glycine max Merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. J Exp Bot 12:3311–3321

Kris-Etherton PM, Hecker KD, Bonanome A, et al (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 9:71–88

Krupa S V. (2003) Effects of atmospheric ammonia (NH3) on terrestrial vegetation: a review. Envionmental Pollut 124:179–221

Lafleur B, Thiffault E, Paré D, et al (2012) Effects of hog manure application on the nutrition

and growth of hybrid poplar (Populus spp.) and on soil solution chemistry in short-rotation woody crops. Agric Ecosyst environmen 155:95–104

Lam V, Watanabe T, Phan T, Khai L (2002) A case study: introduction of low-cost biogas digester to small-scale farming systems

Lampkin N (1990) Organic farming. Farming press books. Ipswich. United Kingdom.

Li B, Li G, Kronzucker HJ, et al (2014) Ammonium stress in Arabidopsis: signaling, genetic loci, and physiological targets. Trends Plant Sci 19:107–114

Li B, Li Q, Xiong L, et al (2012) Arabidopsis plastid AMOS1/EGY1 integrates abscisic acid signaling to regulate global gene expression response to ammonium stress. Plant Physiol 160:2040–2051

Li BH, Shi. WM (2007) Effects of elevated NH4+ on Arabidopsis seedlings different in accessions. Acta Pedofil Sin 44:508–515

Li Q, Li X, Tang B, Gu M (2018) Growth responses and root characteristics of lettuce grown in aeroponics, hydroponics, and substrate culture. Horticulturae 4:35

Liedl BE, Cummins M, Young A, et al (2004) Hydroponic lettuce production using liquid effluent from poultry waste bioremediation as a nutrient source. In VII International Symposium on Protected Cultivation in Mild Winter Climates: Production, Pest Management and Global Competition 659. pp 721–728

Liedl BE, Wilfong K, Taylor C, Mazzaferro K (2006) Liquid effluent from poultry waste bioremediation as a nutrient source for hydroponic cucumber production. HortScience 4:997A – 997

Lin D, Xiao M, Zhao J, et al (2016) An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules 21:1374

Lind OP, Hultberg M, Bergstrand KJ, et al (2020) Biogas Digestate in Vegetable Hydroponic Production: pH Dynamics and pH Management by Controlled Nitrification. Waste and Biomass Valorization 1–11

Liu WK, Yang QC, Du L (2009) Soilless cultivation for high-quality vegetables with biogas manure in China: Feasibility and benefit analysis. Renewable Agriculture and Food Systems,. Renew Agric Food Syst 300–307

Liu WK, Yang QC, du Lian F, et al (2011) Nutrient supplementation increased growth and nitrate concentration of lettuce cultivated hydroponically with biogas slurry. Acta Agric Scand Sect B Soil Plant Sci 61:391–394

Liu X, Ardo S, Bunning M, et al (2007) Total phenolic content and DPPH radical scavenging activity of lettuce (Lactuca sativa L.) grown in Colorado. LWT Food Sci Technol 40:552–557

Liu Y, Lai N, Gao K, et al (2013) Ammonium inhibits primary root growth by reducing the length of meristem and elongation zone and decreasing elemental expansion rate in the root apex in Arabidopsis thaliana. PLoS One 8:e61031.

Liu YJ, Tong YP, Zhu YG, et al (2006) Leaf chlorophyll readings as an indicator for spinach yield and nutritional quality with different nitrogen fertilizer applications. J Plant Nutr 29:1207–1217

Llorach R, Martínez-Sánchez A, Tomás-Barberán FA, et al (2008) Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem 108:1028–1038

Loqué D, Mora SI, Andrade SL, et al (2009) Pore mutations in ammonium transporter AMT1 with increased electrogenic ammonium transport activity. J Biol Chem 284:24988–24995

Loria ER, Sawyer JE, Barker DW, et al (2007) Use of anaerobically digested swine manure as a nitrogen source in corn production. In: Agronomy Journal. pp 1119–1129

Madeira AC, De Varennes A (2005) Use of chlorophyll meter to assess the effect of nitrogen on sweet pepper development and growth. J Plant Nutr 28:1133–1144

Makádi M, Orosz A, Tomócsik V (2012) Digestate: A New Nutrient Source - Review, Biogas, Dr. Sunil Kumar (Ed.), ISBN: 978-953-51-0204-5, InTech, Available from: http://www.intechopen.com/books/biogas/digestate-a-new-nutrient-source-review

Makádi M, Tomócsik A, Kátai J, et al (2008) Nutrient cycling by using residues of bioenergy production - effects of biogas-digestate on plant and soil parameters. Cereal Res Commun 1807–1810

Mampholo BM, Maboko MM, Soundy P, Sivakumar D (2016) Phytochemicals and Overall Quality of Leafy Lettuce (Lactuca sativa L.) Varieties Grown in Closed Hydroponic System. J Food Qual 39:805–815

Man J, Shi Y, Yu Z, Zhang Y (2015) Dry matter production, photosynthesis of flag leaves and water use in winter wheat are affected by supplemental irrigation in the Huang-Huai-Hai Plain of China. PLoS One 10:e0137274.

Mapson LW, Cruickshank EM (1947) Effect of various salts on the synthesis of ascorbic acid and carotene in cress seedlings. Biochem J 41:197-205.

Masarirambi MT, Hlawe MM, Oseni OT, Sibiya TE (2010) Effects of organic fertilizers on growth, yield, quality and sensory evaluation of red lettuce (Lactuca sativa L.) ‘Veneza Roxa.’ Agric Biol J N Am, 1:1319–1324

Massé DI, Talbot G, Gilbert Y (2011) On farm biogas production: A method to reduce GHG emissions and develop more sustainable livestock operations. Anim Feed Sci Technol 436–

445

Mayer-Foulkes DA (2011) A survey of macro damages from non-communicable chronic diseases: Another challenge for global governance. Glob Econ J 11:1–26

Meagy MJ, Eaton TE, Barker A V. (2016) Assessment of mineral nutrient concentrations of lettuce in response to cultivar selection and fertilization in greenhouse production. J Plant Nutr 39:1796–1808

Medicine I of (2005) Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Institute of Medicine, Washington D.C. National Academies Press (US).

Medicine I of (1997) Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Institute of Medicine, Washington, D.C. National Academies Press (US).

Medicine I of (1998) Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Institute of Medicine, Washington, D.C. National Academies Press (US).

Medina E (1984) Nutrient balance and physiological processes at the leaf level. In Physiological ecology of plants of the wet tropics (pp. 139-154). Springer, Dordrecht. pp 139–154

Menardo S, Gioelli F, Balsari P (2011) The methane yield of digestate: effect of organic loading rate, hydraulic retention time, and plant feeding. Bioresour Technol 102:2348–2351

Mihaljević S, Radić S, Bauer N, et al (2011) Ammonium-related metabolic changes affect somatic embryogenesis in pumpkin (Cucurbita pepo L.). J Plant Physiol 168:1943–1951

Miller J, Rucker R (2012) Pantothenic acid. In: Erdman JW, Macdonald IA, Zeisel SH, eds. Present Knowledge in Nutrition. 10th ed. Washington, DC: Wiley-Blackwell:375-90.

Mohankumar JB, Uthira L, Maheswari SU (2018) Total phenolic content of organic and conventional green leafy vegetables. J Nutr Hum Heal 2:1–6

Mollavali M, Perner H, Rohn S, et al (2018) Nitrogen form and mycorrhizal inoculation amount and timing affect flavonol biosynthesis in onion (Allium cepa L.). Mycorrhiza 28:59–70

Möller K, Schulz R, Müller T (2010) Substrate inputs, nutrient flows and nitrogen loss of two centralized biogas plants in southern Germany. Nutr Cycl Agroecosystems 87:307–325

Möller K, Stinner W, Deuker A, Leithold G (2008a) Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. Nutr Cycl Agroecosystems 82:209–232

Möller K, Stinner W, Deuker A, Leithold G (2008b) Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. Nutr Cycl Agroecosystems 82:209–232

Morris MC, Evans DA, Tangney CC, et al (2006) Associations of vegetable and fruit consumption with age-related cognitive change. Neurology 67:1370–1376

Mou B (2009) Nutrient content of lettuce and its improvement. Curr Nutr Food Sci 5:242-248.

Mou B, Ryder EJ (2004) Relationship between the nutritional value and the head structure of lettuce. Acta Hortic 637:361–7

Mulla D, Birr A, Randall G, et al (2001) Impacts of animal agriculture on water quality: technical work paper. Minnesota Environmental Quality Board, Minnesota Planning (Agency), St. Paul.

Nicola S, Hoeberechts J, Fontana E (2005) Comparison between traditional and soilless culture systems to produce rocket (Eruca sativa) with low nitrate content. In International Symposium on Soilless Culture and Hydroponics; International Society for. pp 549–555.

Nicoletto C, Santagata S, Zanin G, Sambo P (2014) Effect of the anaerobic digestion residues use on lettuce yield and quality. Sci Hortic (Amsterdam) 180:207–213

Nicolle C, Carnat A, Fraisse D, et al (2004) Characterisation and variation of antioxidant micronutrients in lettuce (Lactuca sativa folium). J Sci Food Agric 84:2061–2069

Nkoa R (2014) Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. Agron Sustain Dev 34:473-492.

Norenberg MD, Rama Rao K V., Jayakumar AR (2009) Signaling factors in the mechanism of ammonia neurotoxicity. Metab. Brain Dis. 24:103–117

Olsen KM, Lea US, Slimestad R, et al (2008) Differential expression of four Arabidopsis PAL genes; PAL1 and PAL2 have functional specialization in abiotic environmental-triggered flavonoid synthesis. J Plant Physiol 165:1491–1499

Paavola T, Rintala J (2008) Effects of storage on characteristics and hygienic quality of digestates from four co-digestion concepts of manure and biowaste. Bioresour Technol 99:7041–7050

Pan Z, Qi G, Andriamanohiarisoamanana FJ, et al (2018) Potential of anaerobic digestate of dairy manure in suppressing soil-borne plant disease. Anim Sci J 89:1512–1518

Pavia M, Pileggi C, Nobile CGA, Angelillo IF (2006) Association between fruit and vegetable consumption and oral cancer: a meta-analysis of observational studies. Am J Clin Nutr 83:1126–1134

Pellati F, Benvenuti S, Magro L, et al (2004) Analysis of phenolic compounds and radical scavenging activity of Echinacea spp. J Pharm Biomed Anal 35:289–301

Pérez-Jiménez J, Neveu V, Vos F, Scalbert A (2010) Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. Eur J Clin Nutr 64:112–120

Petropoulos SA, Chatzieustratiou E, Constantopoulou E, Kapotis G (2016) Yield and quality of

lettuce and rocket grown in floating culture system. Not Bot Horti Agrobot Cluj-Napoca 44:603–612

Pognani M, D'Imporzano G, Scaglia B, Adani F (2009) Substituting energy crops with organic fraction of municipal solid waste for biogas production at farm level: A full-scale plant study. Process Biochem 44:817–821

Przygocka-Cyna K, Grzebisz W, Lukowiak R, Biber M (2018) Biomass ash and biogas digestate bio-fertilizers as a source of nutrients for light acid soil–an exhaustion test. J Elem 23:413–428

Qi X, Zhang S, Wang Y, Wang R (2005) Advantages of the integrated pig-biogas-vegetable greenhouse system in North China. Ecol Eng 24:175–183

Qin C, Qian W, Wang W, et al (2008) GDP-mannose pyrophosphorylase is a genetic determinant of ammonium sensitivity in Arabidopsis thaliana

Qu H, Madl RL, Takemoto DJ, et al (2005) Lignans are involved in the antitumor activity of wheat bran in colon cancer SW480 cells. J Nutr 3:598–602

Rajashekar CB, Oh M-M, Carey EE (2012) Organic Crop Management Enhances Chicoric Acid Content in Lettuce. Food Nutr Sci 3:1296–1302

Rezk BM, Haenen GR, van der Vijgh WJ, Bast A (2003) Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophores. FEBS Letters. Elsevier 555:601– 605

Roberts S (2018) What Is the Main Function of Phosphorus in the Body? Assessed on: March 06, 2020. Available at: https://healthyeating.sfgate.com/main-function-phosphorus-body-5789.html

Ronga D, Setti L, Salvarani C, et al (2019) Effects of solid and liquid digestate for hydroponic

baby leaf lettuce (Lactuca sativa L.) cultivation. Sci Hortic (Amsterdam) 172–181

Roosta HR, Schjoerring JK (2007) Effects of ammonium toxicity on nitrogen metabolism and elemental profile of cucumber plants. J Plant Nutr 30:1933–1951

Sacks FM, Willett WC, Smith A, et al (1998) Effect on Blood Pressure of Potassium, Calcium, and Magnesium in Women With Low Habitual Intake. Hypertension 31:131–138

Salminen E, Rintala J, Härkönen J, et al (2001) Anaerobically digested poultry slaughterhouse wastes as fertiliser in agriculture. Bioresour Technol 78:81–88

Santamaria P (2006) Nitrate in vegetables: Toxicity, content, intake and EC regulation. J. Sci. Food Agric. 86:10–17

Santos FT, Goufo P, Santos C, et al (2016) Comparison of five agro-industrial waste-based composts as growing media for lettuce: Effect on yield, phenolic compounds and vitamin C. Food Chem 209:293–301

Sapkota S, Sapkota S, Liu Z (2019) Effects of nutrient composition and lettuce cultivar on crop production in hydroponic culture. Horticulturae 5:72

Savvas D, Passam HC, Olympios C, et al (2006) Effects of Ammonium Nitrogen on Lettuce Grown on Pumice in a Closed Hydroponic System

Scaglia B, Pognani M, Adani F (2017) The anaerobic digestion process capability to produce biostimulant: the case study of the dissolved organic matter (DOM) vs. auxin-like property. Sci Total Environ 589:36-45.

Scott J, Rébeillé F, Fletcher J (2000) Folic acid and folates: The feasibility for nutritional enhancement in plant foods. J. Sci. Food Agric. 80:795–824

Seefeldt S (2015) Crop Production and Soil Mangement Series Steven Seefeldt, Extension Faculty, Agriculture and Horticulture. Assessed on: September 14, 2020. Available at:

http://cespubs.uaf.edu/index.php/download_file/1025/.

Shohag MJI, Wei Y, Yang X (2012) Changes of Folate and Other Potential Health-Promoting Phytochemicals in Legume Seeds As Affected by Germination. J Agric Food Chem 60:9137–9143

Silber A, Bar-Tal A (2008) Nutrition of substrate-grown plants. Soil Cult Theory Pract Elsevier, San Diego, CA, 4:291-339.

Slamet W, Purbajanti ED, Darmawati A, Fuskhah E (2017) Leaf area index, chlorophyll, photosynthesis rate of lettuce (Lactuca sativa L) under N-organic fertilizer. Indian J Agric Res 4:365–369

Soetan KO, Olaiya CO, Oyewole OE (2010) The importance of mineral elements for humans, domestic animals and plants: A review. African J food Sci 5:200-222.

Somasundaram E, Amanullah MM, Vaiyapuri K, et al (2007) Influence of organic sources of nutrients on the yield and economics of crops under maize based cropping system. J Appl Sci Res 1774-1777.

Sommer SG, Husted S (1995a) A simple model of pH in slurry. J Agric Sci 124:447–453

Sommer SG, Husted S (1995b) The chemical buffer system in raw and digested animal slurry

Southon S (2000) “Increased Fruit and Vegetable Consumption within the EU: Potential Health Benefits,” Food Research International. 33:211-217.

Squire GR, Ong CK, Monteith JL (1987) Crop growth in semi-arid environment. In: Proceedings of 7th International Workshop, 7-11 April, 1986, International Crops Research Institute for Semi-Arid Tropics. Patancheru, Hyderabad, pp 219-231.

Stinner W, Möller K, Leithold G (2008) Effects of biogas digestion of clover/grass-leys, cover crops and crop residues on nitrogen cycle and crop yield in organic stockless farming

systems. Eur J Agron 29:125–134

Stoknes K, Scholwin F, Krzesiński W, et al (2016) Efficiency of a novel "Food to waste to food" system including anaerobic digestion of food waste and cultivation of vegetables on digestate in a bubble-insulated greenhouse. Waste Manag 466–476

Su YY, Lu Y, Shi WS (2008) Effect of biogas fertilizer on yield and quality of lettuce in soilless culture. [J] Soil Fertil Sci China 1

Sulaiman SF, Yusoff NAM, Eldeen IM, et al (2011) Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (Musa sp.). J Food Compos Anal 1–10.

Survase SA, Bajaj IB, Singhal RS (2006) Biotechnological production of vitamins. Food Technol Biotechnol 44:381–396

Szűcs B, Simon M, Füleky G (2006) Anaerobic pre-treatment effects on the aerobic degradability of wastewater sludge. Proceedings of the Internationale Conference ORBIT 2006, Weimar, 13-15 September, 2006. Part 2, pp. pp 425-434.

Tambone F, Genevini P, D'Imporzano G, Adani F (2009) Assessing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW. Bioresour Technol 100:3140–3142

Tambone F, Scaglia B, D'Imporzano G, et al (2010) Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 5:577–583

Tamilselvi SM, Chinnadurai C, Ilamurugu K, et al (2015) Effect of long-term nutrient managements on biological and biochemical properties of semi-arid tropical Alfisol during

maize crop development stages. Ecol Indic 48:76–87

Tani M, Sakamoto N, Kishomoto T, Umetsu K (2006) Utilization of anaerobically digested slurry combined with other waste following application to agricultural land. Int Congr Ser 1293:331.

Teglia C, Tremier A, Martel JL (2011) Characterization of solid digestates: Part 2, assessment of the quality and suitability for composting of six digested products. Waste and Biomass Valorization 2:113–126

Tian X, Li S, Wang Z, et al (2003) Response of lettuce to different nitrogen forms. Ying yong sheng tai xue bao=. J Appl Ecol 14:377

Tiquia SM, Tam NFY, Hodgkiss IJ (1996) Effects of composting on phytotoxicity of spent pig-manure sawdust litter. Environ Pollut 93:249–256

Tiwari VN, Tiwari KN, Upadhyay RM (2000) Effect of crop residues and biogas slurry incorporation in wheat on yield and soil fertility. J Indian Soc Soil Sci 48:515–520

Trumbo P (2014) Pantothenic acid. In: Ross AC, Caballero B, Cousins RJ, et al., eds. Modern Nutrition in Health and Disease. 11th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2014:351-7.

Tshikalange B, Bello ZA, Ololade OO (2020) Comparative nutrient leaching capability of cattle dung biogas digestate and inorganic fertilizer under spinach cropping condition. Environ Sci Pollut Res 27:3237–3246

USDA (2015) National Nutrient Database for Standard Reference Release 28. USDA, Washington, D.C.

Valverde-Barrantes OJ, Freschet GT, Roumet C, Blackwood CB (2017) A worldview of root traits: the influence of ancestry, growth form, climate and mycorrhizal association on the

functional trait variation of fine-root tissues in seed plants. New Phytol 215:1562–1573

Wargovich MJ (2000) Anticancer Properties of Fruits and Vegetables

Warman PR, Havard KA (1997) Yield, vitamin and mineral contents of organically and conventionally grown carrots and cabbage. Agric Ecosyst Environ 61:155–162

Watson SA, Noggle. GR (1947) Effect of mineral deficiencies upon the synthesis of riboflavin and ascorbic acid by the oat plant. Plant Physiol 22:228–243

Webb AR, Hawkes FR (1985) Laboratory scale anaerobic digestion of poultry litter: Gas yield-loading rate relationships. Agric wastes 13:31–49

Weiland P (2010) Biogas production: current state and perspectives. Appl Microbiol Biotechnol 849–860

Wenke L, Du L, Yang Q (2009) Biogas slurry added amino acids decreased nitrate concentrations of lettuce in sand culture. Acta Agric Scand Sect B Soil Plant Sci 59:260–264

Wright AF, Bailey JS (2001) Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 dry combustion analyzer. Commun Soil Sci Plant Anal 32:3243–3258

Xu WH, Wang ZY, Quan YM, et al (2003a) Effect of application of biogas slurry on nitrate content and nutrition quality of lettuce and romaine lettuce. Rural Eco-Environment, 19:34–37

Xu WH, Wang ZY, Wang Q, et al (2003b) Effects of different biogas slurry on nutrient quality and nitrate content of lettuce. China biogas 21:11–13

Yalovsky S, Bloch D, Sorek N, Kost B (2008) Regulation of membrane trafficking, cytoskeleton dynamics, and cell polarity by ROP/RAC GTPases. Plant Physiol 147:1527–1543

You Q, Chen F, Wang X, et al (2012) Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. LWT-Food Sci Technol 46:164–168

Yu FB, Luo XP, Song CF, et al (2010) Concentrated biogas slurry enhanced soil fertility and tomato quality. Acta Agric Scand Sect B Soil Plant Sci 60:262–268

Zandvakili OR, Barker A V., Hashemi M, et al (2019) Comparisons of commercial organic and chemical fertilizer solutions on growth and composition of lettuce. J Plant Nutr 42:990–1000

Zandvakili OR, Ebrahimi E, Hashemi M, et al (2017) The potential of green manure mixtures to provide nutrients to a subsequent lettuce crop. Commun Soil Sci Plant Anal 48:2246–2255

Zhao X, Carey EE, Young JE, et al (2007) Influences of organic fertilization, high tunnel environment, and postharvest storage on phenolic compounds in lettuce. HortScience 42:71–76

Zuo J, Tang W, Xu Y (2015) Anti-Hepatitis B Virus Activity of Chlorogenic Acid and Its Related Compounds. In Coffee in health and disease prevention (pp. 607-613). Academic Press.

Chapter 2

2. Effects of anaerobic dairy digestate on growth and yield of lettuce in a hydroponic greenhouse production system – a pilot study

2.1. Abstract

Anaerobic digestion is a low-cost waste management method which produces energy and DD)which could be used as a nutrient source for vegetable production. A pilot-scale study was carried out to investigate the effects of DD on growth and yield of lettuce in hydroponic system. Experimental treatments were two lettuce varieties: i) Newham, ii) Romaine, and three nutrient feed solutions: i) nutrient solution (NS), ii) dairy digestaet alone (DD), and iii) combination of both (50% NS + 50% DD). The experiment was laid out in a completely randomized design with factorial arrangements and replicated three times. Results showed that Romaine cultivated in NS produced significantly higher LA chlorophyll content, root dry weight and yield, compared to DD and Newhamwhich produced lower LA, chlorophyll content and yield. Irrespective to lettuce varieties, the results indicated that DD showed 52% and 33% lower LA, 59% and 34% lower root dry weight and 71% and 55% yield reduction than NS and NS+DD treatments, respectively and was negatively correlated with ammonium. Results further showed luxury uptake of NH_4^+-N in DD which was 136% and 52% higher in lettuce grown in DD compared to NS and NS+DD respectively. Lower LA, chlorophyll content, root growth and yield in DD treatment might be due to toxic effects of higher NH_4^+-N, which might have reduced root growth and yield. Romaine variety produced higher LA, chlorophyll content, root dry weight and yield than Newham irrespective of nutrient solution. It can be concluded that DD can be used as organic mineral nutrient source for production of lettuce in hydroponics, considering quality and health benefits of

organically produced lettuce, higher inorganic fertilizer prices, and reduction of carbon foot prints. However, further research is required to reduce NH_4^+: NO_3^- in DD without dilution to avoid nutrient losses and to validate the utilization of DD as a sole mineral nutrients source in hydroponics; although, dilution reduces the NH_4^+-N, other macro and micronutrients and consequently results in reduced growth and significant reduction in yield.

Keywords:

Ammonium toxicity, chlorophyll content, dairy digestate, hydroponics, leaf area, lettuce, root growth, yield

2.2. Introduction

Lettuce (*Lactuca sativa* L.) is a leafy vegetable, contains high amount of minerals, (Squire et al. 1987) phenolics, vitamin C, folates and carotenoids (Nicolle et al. 2004). These phytochemicals are known to enhance immunity and reduce diseases in humans (Qu et al. 2005). Therefore, lettuce intake can be helpful not only to lessen the diseases but also to overcome the food insecurity and malnutrition issues. However, growth, yield and phytochemical profile of lettuce depend on the mineral composition of substrate/solution or growth media, genotypes and environmental conditions. Organic fertilizers may increase the crop growth, yield and may reduce the cost of production (Yu et al. 2010; Makádi et al. 2012). For example, anaerobic digestate is known to enhance sustainable growth and yield and is considered as a good alternative to synthetic chemical fertilizers due to wide range of macro and micro nutrients availability (Makádi et al. 2012).

Anaerobic digestion is a method in which dairy manure, food waste, crop residue and fish waste are decomposed in the absence of oxygen to produce biogas, solid manure and liquid waste slurry (Pan et al. 2018). Biogas is considered as a renewable energy source, whereas, liquid waste slurry is a rich source of macro and micronutrients; and therefore, can be used as soil amendment, or as a bio-fertilizer (Massé et al. 2011). The use of such liquid waste increases the nutrient availability over the undigested slurry, minimizes the pathogenicity, odors and putrescibility (Drennan and DiStefano 2010) and mitigates negative environmental impacts (Fernández-Cirelli et al. 2009; Drennan and DiStefano 2010). Different studies conducted on characterization of dairy digestate (DD) revealed that it contains macro and micronutrients, reduced organic matter and viscosity, high pH, and smaller C:N than undigested animal manures making it potentially a suitable fertilizer (Möller et al. 2008b; Massé et al. 2011). However, presence of higher concentration of NH_4^+ -N in DD could be toxic to plant growth and a threat to the environment (Nkoa 2014). Similarly, due to higher concentrations of nutrients in DD than raw manure (Möller et al. 2008b; Knapp Haraldsen et al. 2011), its land applications can be a potential source of surface and groundwater pollution (Mulla et al. 2001). These concerns prompted the development of alternate methods of using DD for crop production without losing its potential benefits as rich nutrient source. One such alternative method could be utilizing DD in soilless culture or hydroponic system to mitigate environmental concerns while making use of DD as a sustainable and alternative biofertilizer source to enhance crop growth and yield.

Hydroponics is a method of growing plants in a water based nutrient rich solution without use of the soil, instead the root system is supported using an inert medium i.e. perlite, rock wool, peat moss, clay pellets, or vermiculite (IPL 2020). The basic philosophy is to allow the direct contact of plants roots with the nutrient solution, while also having access to oxygen, which is essential for proper growth (Fullbloom 2020). Additionally, vegetables grown in hydroponic systems grow faster, produce higher yield, require less space, contains higher nutritional value and more desirable sensory attributes compared to soil based production system (Buchanan and Omaye

2013). Lind et al. (2020) investigated the biogas digestate as a fertilizer for production of bok choy (*Brassica rapa* var. chinensis) in hydroponic system and observed similar yields in biogas digestate-based hydroponics and synthetic fertilizers application (Buchanan and Omaye 2013). They concluded that biogas digestate is a suitable plant nutrient source for hydroponic production of bok choy, considering productivity and circularity aspects. Ronga et al. (2019) investigated the nine combinations of solid and liquid digestate with agriperlite, standard solution, soil, peat moss and pelleted digestate for the production of baby leaf lettuce in hydroponic systems in three different experiments. They observed higher plant growth (root and shoot), yield and chlorophyll content in digestate solution compared to other treatment combinations. Wenke et al. (2009) investigated the effects of digestate with different dilutions of inorganic nutrient solution on the lettuce growth and yield. They reported that digestate application significantly reduced the nitrate concentration in lettuce shoot and enhanced yield compared to inorganic nutrient solution and therefore, digestate could be used as a biofertilizer to replace inorganic fertilizers solution in hydroponic systems. However, study conducted by Zandvakili et al. (2019) reported contrary results; they observed lower biomass, mineral nutrient concentration and nitrate contents in lettuce grown in organic liquid solution compared to inorganically grown lettuce. However, Liedl et al. (2006) observed less cucumber yield but higher grade fruit quality in anaerobic poultry digestate solution used in hydroponics compared to inorganic commercial fertilizers. They recorded, digestate fed cucumber produced 7% higher grade fruits compared than inorganic fertilizers. Barzee et al. (2019) evaluated the application of digestate biofertilizers on tomatoes production. They reported that the tomato plants grown in dairy manure digestate produced more tomato yield compared to food waste digestate and synthetic nitrogen fertilizer. As reported above, very few agronomic studies have been conducted on aerobic and anaerobic digestate (Goddek et al. 2016;

Stoknes et al. 2016), specifically information regarding the use of DD as a fertilizer in hydroponic vegetable production system is very limited (Liedl et al. 2006).

Hence, we hypothesized that DD would enhance growth, and yield of lettuce in hydroponic system. To test this hypothesis, we conducted a greenhouse experiment in hydroponics system with the following objectives:

I. to evaluate the effects of DD, NS alone and combine application of DD and NS (50% DD + 50% NS) on the growth and yield of lettuce genotypes
II. to determine the uptake of total N, ammonium-N and nitrate-N in lettuce tissues grown in DD, NS alone and combination of both DD and NS (50% DD + 50% NS) solutions

2.3. Material and Methods

2.3.1. Raising of nursery and plant growing conditions

Lettuce seeds of the Newham and Romaine varieties purchased from High Mowing Organic Seeds (Wolcott, VT, USA) weresown in pre-soaked nursery trays containg peat pellets (Jeffy-7, Lorain, OH, USA). The peat pellets were kept moist to enhance germination and seedling development according to supplier's instructions. Then, trays were placed in a growth chamber (Biochambers, MB, Canada), located on Grenfell Campus, Memorial University of Newfoundland (MUN), Canada. The growth chamber was equipped with a climate control system; growth conditions were 14/10 h day/night duration with 21/19 °C temperature, 80% relative humidity, and 400 – 600 ppm CO_2. One-week old lettuce nursery plants were transplanted in a greenhouse located at St. David's (latitude 51.8812° N, longitude 5.2660° W), NL, Canada. The greenhouse growth conditions were 25/17 °C day/night temperature, 75 - 80% relative humidity and 400 – 600 ppm CO_2 (Zandvakili et al. 2019). These growth conditions were monitored by an in-built Hobo meter (MX1102A, USA) during the execution of experiment. The experimental treatments were three nutrient solution; NS (modified Hoagland solution); 100% DD; and 50% NS + 50% DD (onwards denoted as NS+DD) and two lettuce varieties (Newham and Romaine). The experiment was laid out in a complete randomized design with factorial arrangement and replicated thrice.

2.3.2. Preparation of DD and other nutrient solutions

NS was prepared following the method of Hoagland and Arnon (1950). The DD was sourced from anaerobic digester of New World Dairy Inc. St. David, NL; DD sample was sent to Agriculture and Food Laboratory, University of Guelph, Ontario, to assess the nutrient and heavy metal profile. The DD analysis report showed very high amount of NH_4^+-N (Table 3), which could be toxic to roots and shoot growth of lettuce. Consequently, DD was diluted 10 times to reduce NH_4^+-N concentration in solution to avoid NH_4^+ toxicity. DD solution was prepared in a 1000 L plastic tank following 1:10 ratio of DD and water. Combination of NS and DD treatment (50% NS + 50% DD) was prepared considering 50% N from DD and 50% N from NS. Analyses report of all nutrient solutions is given in Table 2. DD and DD + NS treatments showed variation in pH compared to NS during entire growth period (Figure 1). However, the pH of feed solution was maintained between 5.8 – 6.2 by adding nitric acid (HNO_3).

Table 2: Physiochemical characterization of feed solution used in this study.

	NS			NS+DD			DD		
	Report 1	Report 2	Report 3	Report 1	Report 2	Report 3	Report 1	Report 2	Report 3
pH	5.9	6	5.9	6	5.9	6	6	6.1	6.1
EC (dS m^{-1})	2.47	2.51	2.50	3.7	3.65	3.73	4.1	4.2	4.2
					(mg L^{-1})				
NO_3^-N	196	212	205	72	207	40.3	16.2	149	1.47
NH_4^+-N	8.64	8.24	9.61	122	142	191	292	300	396
P	52	56	59	26	27	34	91	46	54
K	370	384	390	190	270	280	250	230	250
Ca	225	220	230	110	190	170	220	110	130
S	98	101	110	24	34	41	35	27	34
Mg	47	51	48	28	26	25	92	45	42
Na	42	56	53	80	88	100	140	120	150
Fe	5.7	5.6	5.8	2.9	4.2	5.8	8.2	4.1	5.7
Cu	0.1	0.1	0.1	0.63	0.31	0.73	2.9	1.4	1.5
Mn	0.98	1.7	1.2	0.67	0.87	1	2.3	0.82	0.95
Zn	0.5	0.5	0.59	0.85	0.81	1.6	2.7	1.7	2.6

The feed solutions were analyzed on July 04 (7 days after transplanting (DAT)), August 28 (24 DAT), and October 03 (40 DAT) during 2019 and designated as Report 1, 2 and 3, respectively.

Table 3 Minerals and heavy metal concentration in concentrated DD (before dilution)

	Mineral concentration		Heavy metal concentration (mg/kg dry)	Maximum allowable rates (ppm)
Ammonium-N (mg/kg wet)	2200	Lead	0.93	500
Total Kjeldahl Nitrogen (% Wet)	0.355	Arsenic	0.7	75
NO_3 and NO_2 in sample (mg/kg wet)	1.57	Cadmium	0.24	20
Phosphorus (% Wet)	0.0508	Chromium	8.6	1060
Potassium (% Wet)	0.216	Mercury	0.02	5
Zinc (mg/kg dry)	390			
Sodium (% wet)	0.0717			
Copper (mg/kg dry)	650			
Molybdenum (mg/kg dry)	4.8			
Nickel (mg/kg dry)	9.6			
Cobalt (mg/kg dry)	1.7			

*Canadian Council of Ministers of the Environment guidelines (CCME)

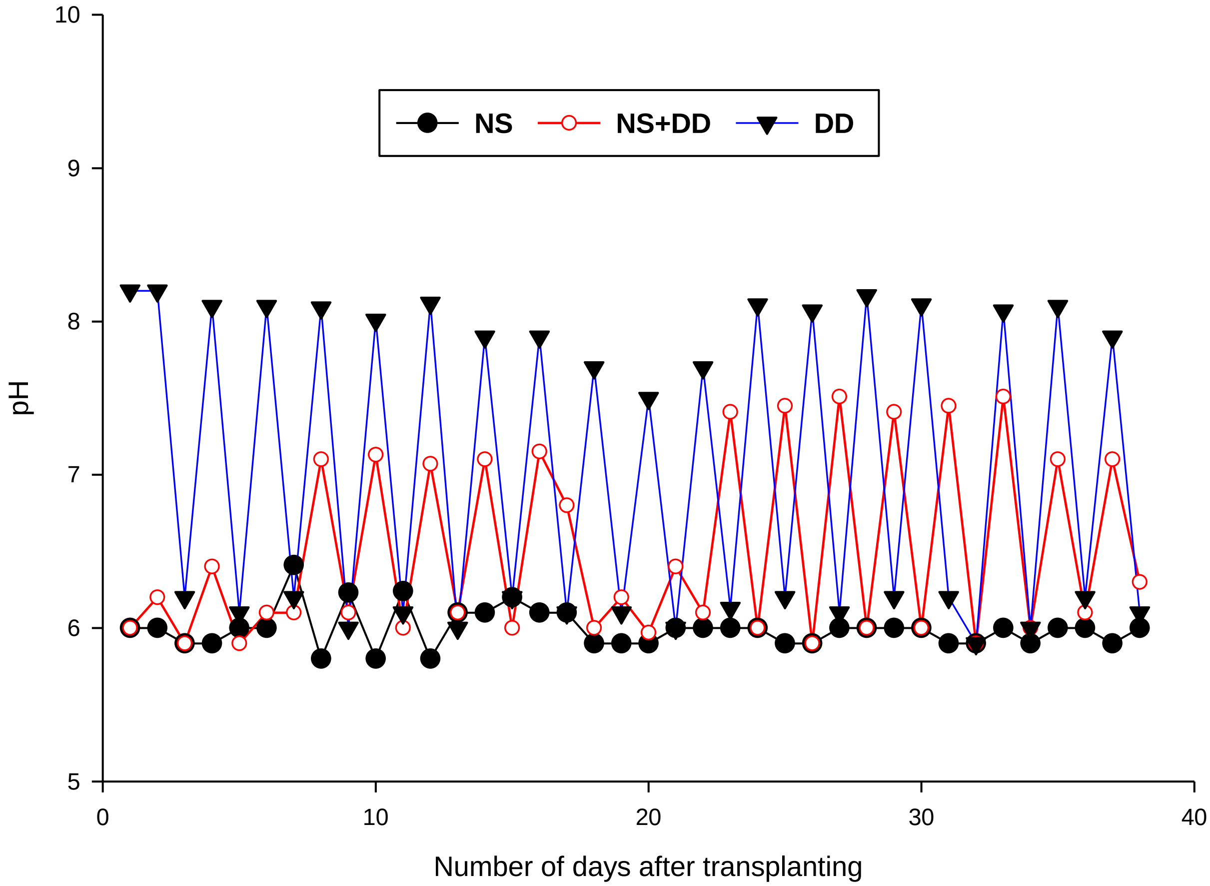

Figure 1: Temporal variation in mineral feed solution's pH used in hydroponics to grow lettuce in greenhouse settings. NS = nutrient solution; NS+ DD= 50% NS + 50% DD; DD= 100% dairy digestate

2.3.3. Crop experiment

Two sets of experiments were conducted in the greenhouse to evaluate the effect of three mineral feed solutions (MFS) on the growth and yield of hydroponically grown lettuce genotypes. One-week-old lettuce plants were transplanted in small opaque water containers (28.60 cm × 37.60 cm × 20.40 cm, 15L capacity) purchased from a local store. A styrofoam sheet was used as a lid on each container to hold four lettuce plants in each container. Uniclife aquarium air pumps (4Watt, 4-LPM, Pressure: 0.016 Mpa) with an adjustable flow rate of 76 to 379 L min^{-1} were purchased and connected to each water container to supply oxygen to lettuce roots using a bubbling stone.

Four plants were transplanted in each container using styrofoam without disturbing the plant roots. There were total 36 plants in 9 containers, 3 containers for each treatment and replicated thrice. Electrical conductivity (EC) and pH of all MFS were monitored 2-3 times daily using a portable EC/pH meter (Accumet© AP85; Fisher Scientific, Hampton, NH, USA) and MFS were replaced weekly with full strength fresh solutions.

2.3.4. Measurements of crop growth parameters and yield

Leaf area - LA (cm^2), total chlorophyll content, root dry weight and yield of two lettuce varieties were measured at harvest (45 days after sowing - DAS). Briefly, LA of randomly selected two plants were measured with portable LA meter (LI-3000C - LI-COR Biosciences, Lincoln, NB, USA), same plants were harvested and taken to the boreal ecosystem research facility (BERF), Grenfell Campus, MUN to determine chlorophyll content and other growth parameters. Plants were then separated into roots and shoots to determine fresh and dry weight (g $plant^{-1}$ FW). The roots were then oven dried (Shell Labs, USA) at 65 °C till a constant weight and final roots dry weight was measured using the weighing balance (RADWAG, PS 6000/C/2, Poland). Finally, yield was measured using the same weighing balance.

Chlorophyll content (Chl a & Chl b) were determined from the same fresh plant leaves following the method of Arnon (1949). Briefly, 0.2 g fresh lettuce leaves were extracted in aqueous 80% acetone. Extracted samples were then centrifuged for 10 min at 2000 rpm. The whole process was performed in a place with low light to decrease the effects of light on chlorophyll concentration. The spectrophotometer was adjusted to zero using the 80% acetone prior to run the samples. Centrifuged samples were then carefully transferred into the cuvettes without disturbing the pellet and concentration of Chl a & Chl b was quantified by determining the extinction of the extract at the major red absorption (QY) maxima of Chl a (~663 nm) and b (~645 nm) and then values were used into the following equations to determine total chlorophyll content in plant leaves.

$$Chl\ a = 12.70\ A663 - 2.69\ A645$$

$$Chl\ b = 22.90\ A645 - 4.68\ A663$$

$$Total\ Chl = Chl\ a + Chl\ b$$

2.3.5. Determination of ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) in lettuce leaves

Nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) in lettuce leaves were determined following the method of Bottoms et al. (2012). Briefly, 3 g of homogenous lettuce leaf tissue was weighed into a 125 mL Erlenmeyer flask and extracted in 15 mL of KCl (2 M). Flasks were shaken for 1 h on a reciprocating shaker (Innova 2300, New Brunswick, 120 V). Solution was removed from the shaker and left undisturbed for 30 min so that plants particles settled down then filtered through a Whatman #1 filter paper into a new bottle (Zandvakili et al. 2017). NO_3^--N and NH_4^+-N were analyzed using QuickChem QC8500 Automated Ion Analyzer, (Lachat Instruments Inc., Loveland, CO, USA) (Bottoms et al. 2012). The concentration of NO_3^--N and NH_4^+-N in sample extracts was determined based on the standard curve developed from 200 mg N/L NH_4^+ stock solution of potassium chloride and ammonium chloride for NH_4^+-N and 200 mg N/L potassium nitrate stock solution for NO_3^-N. These values were used to determine NO_3^- and NH_4^+ uptake in plants following the equation (Environmental Protection Agency 1979; Fishman and Friedman 1989).

Plant NO_3^--N and NH_4^+-N in lettuce was calculated by;

$$NO_3^- - N \text{ or } NH_4^+ - N \text{ in plant (mg N kg}^{-1} \text{ of sample)}$$
$$= NO_3^- - N \text{ or } NH_4^+ - N \text{ in plant} \times \text{extracted volume (15 mL)} \times \text{dilution factor}$$

2.4. Statistical analysis

Two-way analysis of variance (ANOVA) was employed to determine the effects of experimental treatments on lettuce growth, yield and N concentrations (NO_3^--N and NH_4^+-N) in leaves using the Statistix-10 (Analytical Software, FL, USA). Where treatment effects were significant, the means were compared with Fisher's Least Significant Difference (LSD) at alpha 0.05. Prior to data analysis, normality of the data set was tested using Shapiro-Wilkes test. Figures were prepared using SigmaPlot 13.0 software program (Systat Software Inc., San Jose, CA, USA).

2.5. Results

2.5.1. Crop growth parameters

Lettuce genotypes, mineral feed solutions and their interaction had significant effects on leaf area, total chlorophyll content as well as root growth (Figure 2A-C). Interaction between lettuce varieties and mineral feed solution (MFS) had significant ($p<0.05$) effects on LA. Romaine variety and NS treatment produced significantly higher LA (3087.01 cm^2 $plant^{-1}$), followed by Newham and NS treatment compared to the lowest (1111.40 cm^2 $plant^{-1}$) observed in Newham cultivated in DD solution (Figure 2A). Romaine produced significantly higher LA than Newham in DD and NS+DD treatments, likewise, NS+DD treatment produced 39% higher LA than DD treatment (Figure 2A). Interaction between lettuce varieties and MFS had significant ($p<0.05$) effects on total chlorophyll content (Figure 2B). Romaine and NS exhibited higher total chlorophyll content

(46.42 mg g^{-1} FW), followed by Newham and NS treatment compared to the lowest (33.14 mg g^{-1} FW) noted in Newham cultivated in DD solution (Figure 2B). Interestingly, both Romaine and Newham lettuce varieties produced statistically similar chlorophyll content when grown in NS+DD and DD treatments; DD treatment produced lowest chlorophyll content in both varieties compared to NS+DD treatment, though total chlorophyll content in both varieties were statistically similar to each other (Figure 2B).

Statistical analyses indicated significant ($p<0.05$) interaction between MFS and lettuce varieties on root dry weight (Figure 2C). Romaine and NS produced significantly higher root dry weight (3.84 g $plant^{-1}$) followed by Newham with NS (2.88 g $plant^{-1}$) (Figure 2C). However, Newham produced lowest root dry weight (1.03 g $plant^{-1}$) when grown in DD solution. Romaine variety exhibited higher root dry weight in DD, and NS+DD treatments compared to Newham which didn't perform well in all treatments (Figure 2C).

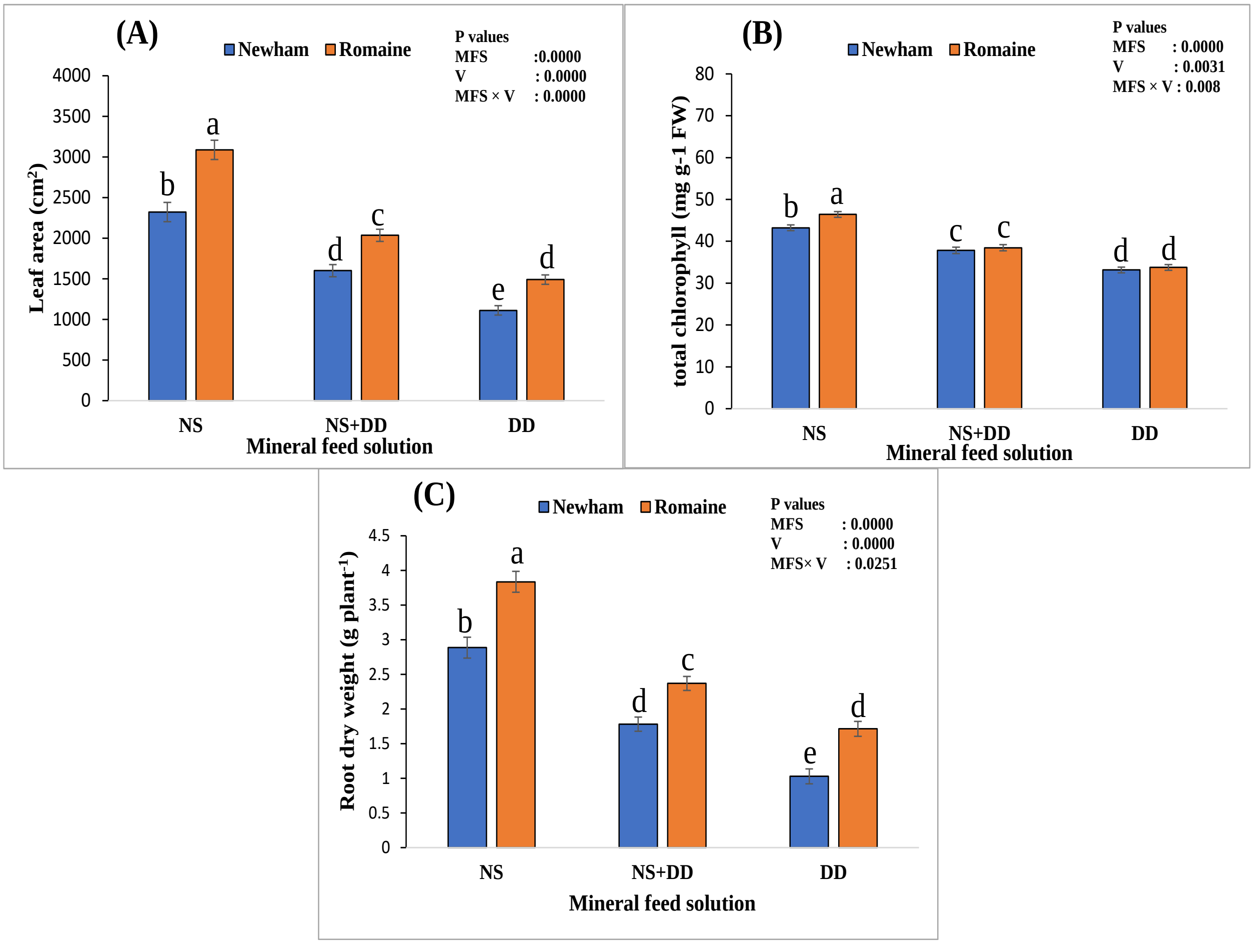

Figure 2: Effects of DD and inorganic nutrient solution on leaf area (A), total chlorophyll (B) and root dry weight (C) of two lettuce varieties under hydroponic cultivation. Vertical bars are means of three replications ±SE. Bars sharing the same letter, for individual factors and interaction, do not differ significantly at LSD ≤ 0.05; NS = nutrient solution; NS+DD = 50% NS + 50% DD; DD = dairy digestate; MFS= mineral feed solution; V = varieties; MFS × V = interaction between mineral feed solution and varieties

Figure 3: Response of Newham (a) and Romaine (b) varieties to root growth cultivated in different mineral feed solutions (MFS) before harvest.

2.5.2. Yield (g plant $^{-1}$)

Lettuce varieties and MFS had significant ($p<0.05$) effects on lettuce yield (Figure 4). Romaine with NS produced significantly higher yield (410.2 g plant $^{-1}$), followed by Newham with NS, though statistically on par with Romaine grown in NS+DD treatment. Newham with DD treatment produced the lowest yield (89.07 g plant^{-1}). Romaine variety showed superior agronomic performance than Newham and produced significantly higher yield irrespective of MFS treatments (Figure 4; Figure 5). Newham could not perform well in all treatments (Figure 4).

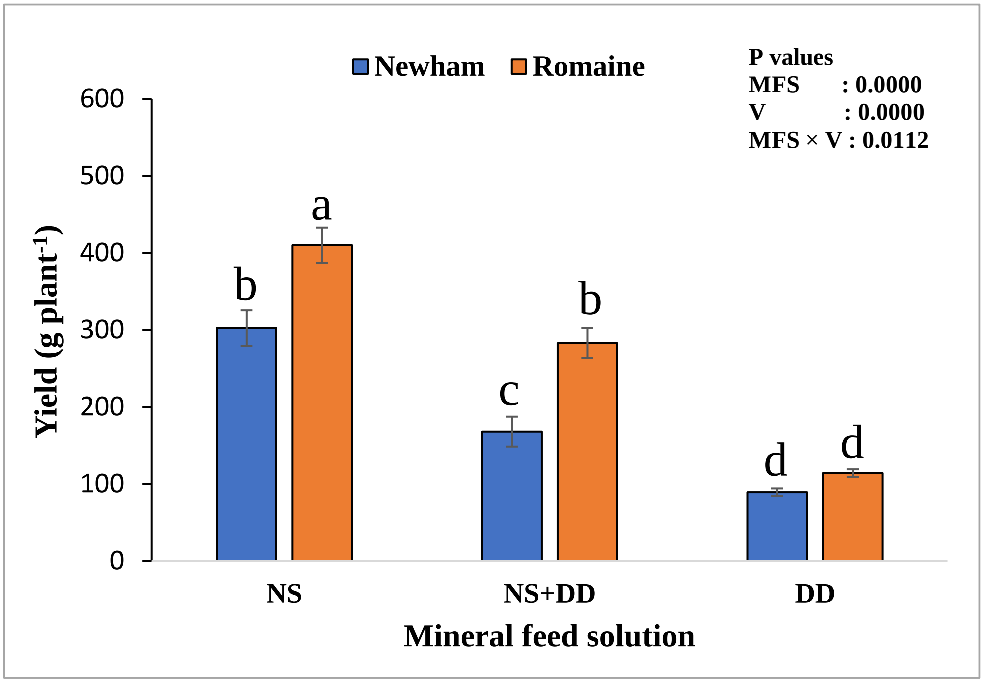

Figure 4: Effects of DD and inorganic nutrient solution on yield of two lettuce varieties tested in hydroponic system. Vertical bars are means of three replications ±SE. Bars sharing the same letter, for individual factors and interaction, do not differ significantly at LSD ≤ 0.05; NS = nutrient solution; NS+DD = 50% NS + 50% DD; DD = dairy digestate; MFS= mineral feed solution; V = varieties; MFS × V = interaction between mineral feed solution and varieties

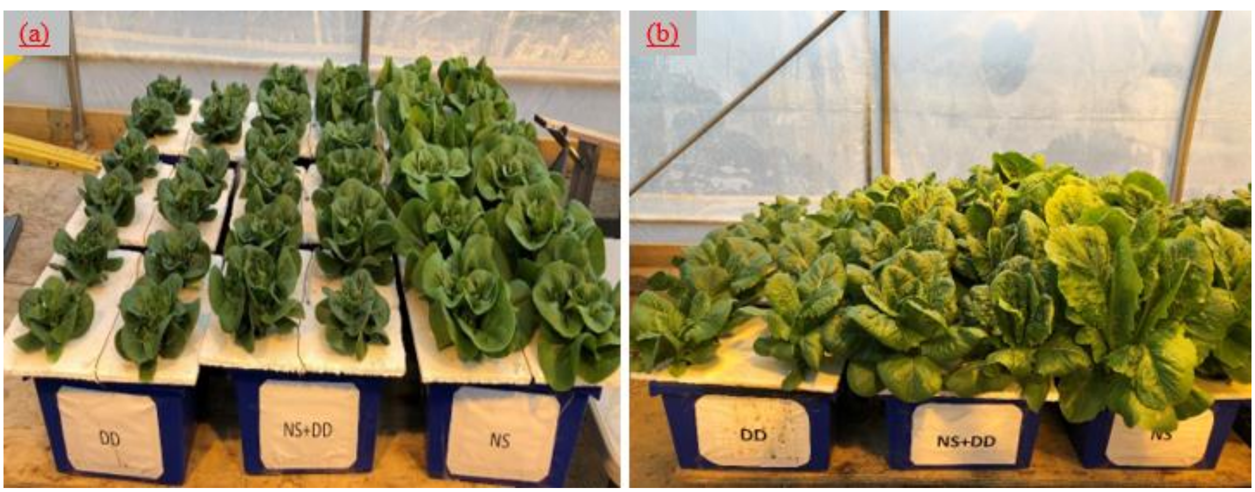

Figure 5: Growth performance of lettuce varieties, Newham (a) Romaine (b) cultivated in different nutrient solution.

2.5.3. Nitrate and ammonium uptake in lettuce leaves (mg N kg^{-1})

Interaction between the lettuce varieties and MFS had significant ($p<0.05$) effects on the uptake of NO_3^--N by lettuce leaf (Figure 6 A). Results indicated that Romaine with NS exhibited higher leaf NO_3^--N (720.83 mg N kg^{-1}), followed by Romaine with NS+DD treatment (513.85 mg N kg^{-1}). Newham treated with DD solution exhibited the lowest NO_3^--N (338.38 mg N kg^{-1}). Newham showed significantly lower leaf NO_3^--N uptake in all MFS treatments compared to Romaine which exhibited much higher NO_3^--N uptake (Figure 6 A). However, the overall NO_3^--N uptake ranges from 338.38 to 720.83 mg N kg^{-1} in all treatments. Statistical analysis further revealed non-significant interactive effects between varieties and MFS on NH_4^+-N concentration in lettuce leaves (Figure 6 B). However, varieties and MFS had significant effects on NH_4^+-N concentration in lettuce leaves. Romaine showed 12% higher NH_4^+-N uptake than Newham. As expected, lettuce grown in DD solution showed higher NH_4^+-N (591.90 mg N kg^{-1}) uptake, followed by NS+DD (390.65 mg N/kg), and the lowest uptake (248.10 mg N kg^{-1}) was observed with NS treatment (Figure 6 B).

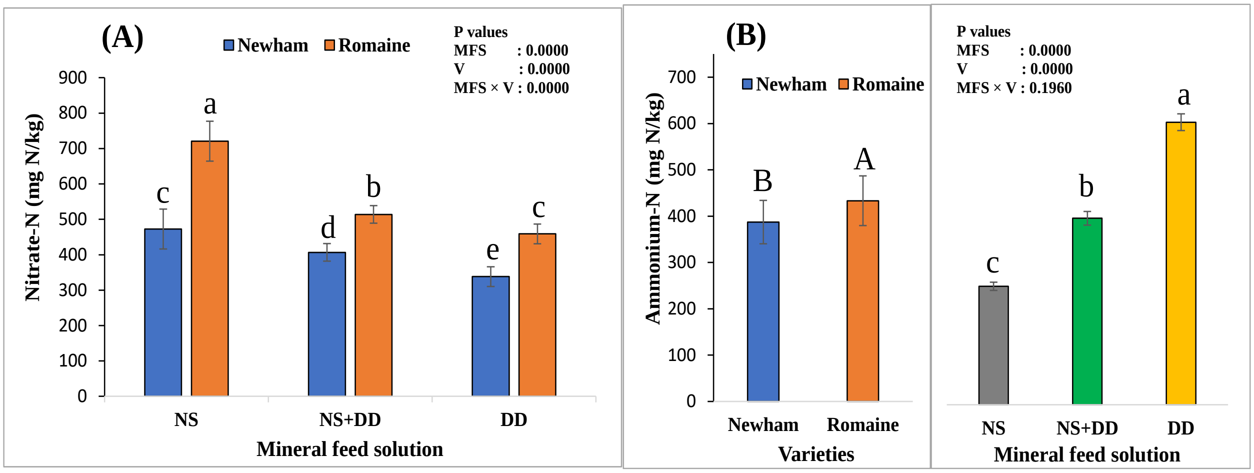

Figure 6: Effects of DD and inorganic nutrient solution on nitrate-N (A) and ammonium-N (B) of lettuce in hydroponic system. Vertical bars are means of three replications ±SE. Bars sharing the same letter, for individual factors and interaction, do not differ significantly at LSD ≤ 0.05; NS = nutrient solution; NS+DD = 50% NS + 50% DD; DD = dairy digestate; MFS= mineral feed solution; V = varieties; MFS × V = interaction between mineral feed solution and varieties

2.6. Discussion

Plant growth is most often limited due to non-availability of resources, particularly water and essential nutrients in the growing medium. LA is an important parameter that determines light interception (Koester et al. 2014), photosynthesis and other physiological processes which leads to enhance the crop yield (Man et al. 2015). mineral feed solution (MFS) plays an important role in enhancing the LA of hydroponically grown lettuce. For example, Medina (1984) observed that N and P played a significant role in enhancing LA and consequently increased photosynthesis. In present study, NS treatment produced higher LA and DD treatment produced the lowest LA of both lettuce varieties (Figure 2 A). This increment in LA might be attributed to readily available mineral nutrients in NS specially NO_3^--N that enhanced LA, contrary to lower NO_3^--N and other macro and micronutrients in DD which reduced LA of lettuce (Hariadi et al. 2016) (Table 2). A previous study conducted by Zandvakili et al. (2019), reported that lettuce plants grown with chemical fertilizers produced higher LA compared to lower LA with liquid organic fertilizers. These studies confirmed the findings of this study.

Chlorophyll content play an important role in energy harvesting reaction during the photosynthesis process that help in CO_2 fixation in the presence of light and maximizing the carbohydrates for energy production (Slamet et al. 2017) and subsequent plant growth. We observed higher chlorophyll content in lettuce leaves grown with NS treatment and the lowest were recorded in DD treatment during both crop trials (Figure 2 B). The higher chlorophyll content of lettuce grown in NS might be due to adequate NO_3^--N and NH_4^+-N and other essential nutrients supply, contrary to predominant NH_4^+-N and low supply of other macro and micronutrients in the DD solution (Table 2). Studies conducted by Zandvakili et al. (2019) and Madeira and De Varennes (2005) also reported higher chlorophyll content in lettuce when grown in Hoagland solution which could be attributed to optimum and consistent supply of N, since N is a constituent of chlorophyll molecule whereas, lower NO_3^--N in DD resulted in reduced leaf chlorophyll content (Figure 2 B).

Plants grown in predominant NO_3^--N solution showed higher root growth, root mass and highly branched roots system than higher NH_4^+-N fed plants (Garbin and Dillenburg 2008). At the cellular level, NH_4^+-N is a fundamental substrate for amino acid and protein synthesis in all living organisms, but it is toxic to cells when present in excess (Norenberg et al. 2009). Similar results were reported in previous studies where stunted root growth was noted in high NH_4^+-N concentration in root zone and root growth inhibition is the key phenotypic indicator of NH_4^+ toxicity in plants (Britto and Kronzucker 2002; Li et al. 2014). Liu et al. (2013) also observed significant reduction in roots growth of *Arabidopsis thaliana* due to higher NH_4^+-N in nutrient solution which might impacted the meristem cells during the mitosis which reduced root growth.

In the present study, we also observed stunted root growth and the lowest root dry weight due to higher NH_4^+-N in DD and NS+DD treatments compared to NS treatment (Figure 2 C & 3; Table 2). Pearson correlations showed significantly negative correlation (r = -0.69**) between the root dry weight and NH_4^+-N concentration in MFS (Figure 7 C). In another study conducted by Qin et al. (2008) observed root growth inhibition in *Arabidopsis thaliana* (mutant based root length assay). They reported that GDP- mannose-pyrophosphorylase (GMPase) enzyme synthesizes GDP-mannose which is in turn essential for the biosynthesis of N-glycoprotein and L-ascorbic acid (AsA) (Conklin et al. 1999a). The GMPase activity in hsn1 and vtc1 mutants are NH_4^+ sensitive (Qin et al. 2008). Of the affected functions, defective protein glycosylation in roots, rather than decreased AsA synthesis, has been linked to the hypersensitivity response (Qin et al. 2008; Barth et al. 2010). This defective protein N- glycosylation which is present in vtc1-1 contributes to cell wall and cell cycle defects, errors in protein folding and cell death in root cells which is directly associated with root growth inhibition mediated by the high NH_4^+ (Qin et al. 2008; Kempinski et al. 2011).

Crop yield depends on several factors such as soil fertility or mineral nutrient composition, genotypes or species and environmental variables (Sapkota et al. 2019). In our study, Romaine lettuce produced higher yield in NS treatment, whereas, Newham produced the lowest yield in DD treatment solution (Figure 4). Higher yield of Romaine cultivated in NS treatment could be attributed to a balanced NO_3^-:NH_4^+, adequate and consistent supply of essential nutrients and genetic potential of Romaine, contrary to the lowest yield of Newham grown in DD treatment solution (Table 2). Various studies have reported that digestate comprehends diverse phytohormones such as auxins, indole acetic acid, gibberellins and other bioactive compounds which are dissolved in organic matter having the potential to promote plant growth and increasing their tolerance against worst conditions (Liu et al. 2009; Scaglia et al. 2017). Our results corroborate the findings of Zandvakili et al. (2019) and Barker et al. (2017), where authors reported higher yields of lettuce and cabbage grown with chemical fertilizer than with organic fertilizers. Li et al. (2018) reported that hydroponics lettuce varieties and MFS composition has a great impact on yield. Authors observed 'Nenglv naiyou' and 'Dasusheng' lettuce varieties produced 88.8 g and 96.1 g fresh weight per plant grown in inorganic MFS containing 210 mg N L^{-1}, suggesting the effect of genotypic variation on yield. We observed similar response of Romaine and Newham lettuce yields grown in NS, NS+DD and DD treatment solutions. Romaine variety showed superior agronomic performance and produced significantly higher yield even with higher NH_4^+- N toxicity stress (DD treatment), whereas, Newham could not perform well under similar conditions. This could be due to better resilience, adaptation and genetic potential of Romaine variety under higher NH_4^+- N stress. Moreover, various species showed varied preferences to N sources, some species preferred NH_4^+ as N sources (Britto and Kronzucker 2002), others such as tomato and cucumber prefer NO_3 source (Roosta and Schjoerring 2007), and some other species prefer combine application of both N sources (NO_3^- and NH_4^+) over the sole source (Errebhi and Wilcox 1990). Higher LA and chlorophyll content of lettuce enhanced yield when cultivated in NS treatment (Figure 7 a & b). Loqué et al. (2009) reported that the gene AMOS1/EGY1 (Plastid metalloprotease), that synthesizes chlorophyll content, dependents on plastid signaling pathway, which is required for the expression of NH_4^+ responsive genes and the maintenance of chloroplast functionality. Chloroplast is directly linked with plant photosynthesis process. Under NH_4^+ stress, the chloroplast receives the stress signal (the plasma membrane acting as the first site of perception of NH_4^+ stress), and triggering of AMOS1/EGY1-dependents retrograde signaling and recruiting downstream abscisic acid (ABA) signaling, to regulate the expression of NH_4^+ which possibly affect the plant growth and ultimately the yield (Li et al. 2012). High NH_4^+- N in DD and NS+DD solution treatments in the present study significantly affected root growth and resulted the lowest LA, chlorophyll content and the final yield (Figure 3 & 7; Table 2).

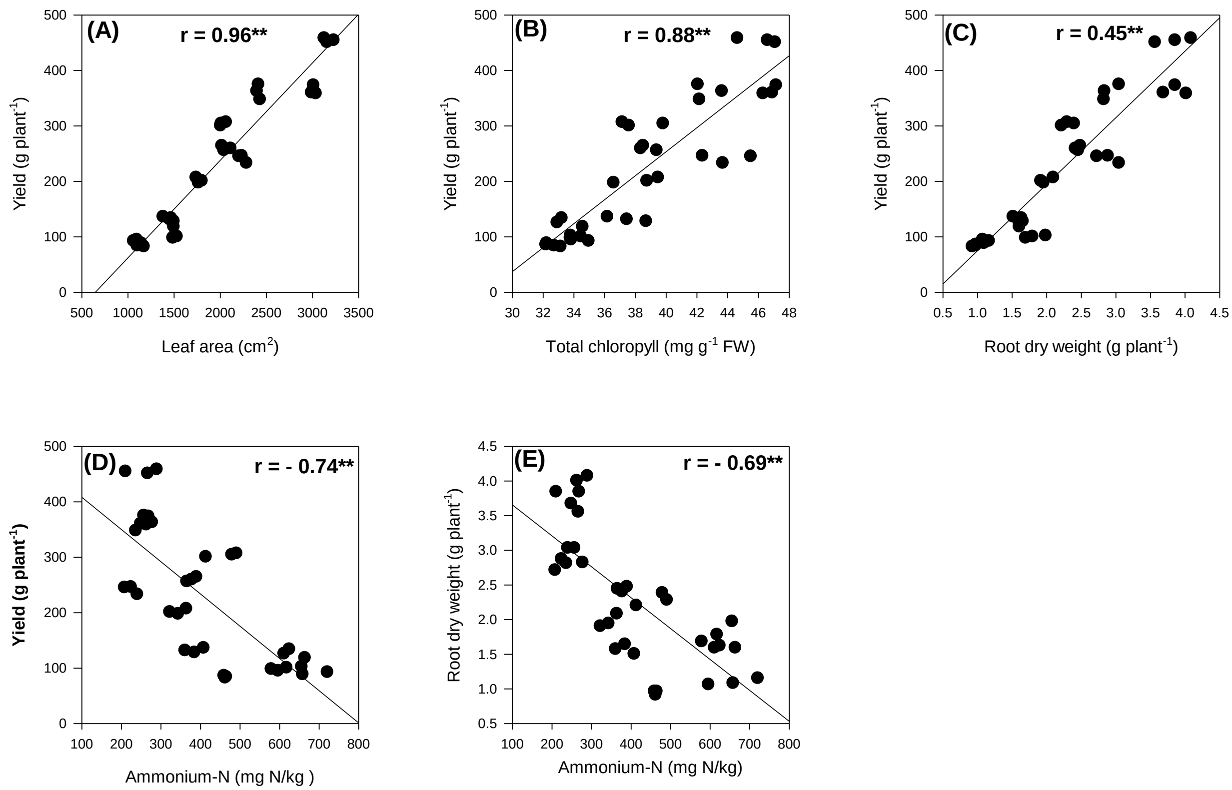

Figure 7**:** Pearson correlation showing the strong positive association between the yield and LA (A), yield and total chlorophyll content (B), yield and root dry weight (C), and strong negative association between yield and ammonium-N (D) and root dry weight and ammonium-N (E) of lettuce when grown in hydroponics system in greenhouse settings.

* significant at $p \leq 0.05$; ** significant at $p \leq 0.01$

Inorganically grown lettuce exhibited higher NO_3^--N concentration (922.7 mg kg^{-1}) in the compared to organically grown lettuce which showed lower NO_3^--N concentration of 208.8 mg kg^{-1} (Wenke et al. 2009). In present study, we also observed that lettuce grown in NS treatment showed higher NO_3^--N (596.79 mg N kg^{-1}) than NS+DD (460.24 mg N kg^{-1}) and DD (398.69 mg N kg^{-1}) treatment solutions, respectively (Figure 6 A), however, NO_3^--N concentration is much lower than that was reported by Wenke et al. (2009). Lower NO_3^--N in DD might be due to the nitrogen that was bound with organic material and released nutrients slowly affecting the nitrate accumulation in plants (Hassan et al. 2012). High NO_3^--N in leafy vegetables impairs the oxygen supply in human body due to nitrite and hemoglobin interaction (Santamaria 2006). Additionally, NO_3^- has the potential to combine with secondary amines which leads to nitrosamines and cause gastrointestinal cancer (Hord et al. 2009a).

As expected, we observed higher concentration of NH_4^+-N in lettuce leaves grown in DD treatment compared to NS treatment in the present study (Figure 6 B; Table 2). Our results are in line with Hoque et al. (2007), who observed higher NH_4^+-N (2100 mg kg^{-1}) in lettuce grown in higher NH_4^+-N solution, whereas, we observed 600 mg N kg^{-1} in lettuce grown in DD solution. Ammonium-N is usually present in high amounts in the organic-based fertilizer (Möller et al. 2008b) and is very detrimental for plant growth and development (Li et al. 2014). Additionally, lettuce roots have more affinity to uptake NH_4^+-N than NO_3^- -N (Tian et al. 2003) which might be the main reason for higher NH_4^+-N in the leaves of lettuce.

2.7. Conclusion

Nutrients availability and balance is very important for growth, development and yield of crops especially when grown in hydroponic systems. Lettuce plants grown in standard nutrient solution (NS) showed higher LA, chlorophyll content, root dry weight, and yield. However, when dairy digestate (DD) was used as a sole mineral nutrient source for lettuce production, it resulted in lower LA, chlorophyll content, root dry weight and final yield. These reduced growth parameters could be associated with high NH_4^+: NO_3^- in DD treatment solution which showed toxic effects on root growth which reduced the essential nutrients uptake and consequently resulted in reduced final yield. Romaine produced significantly higher plant growth parameters and yield compared to

Newham irrespective of Mineral feed solution under hydroponic systems with tested treatments. It can be concluded that DD could be used as a sustainable biofertilizer source for production of hydroponic lettuce if NH_4^+: NO_3^- is reduced without diluting DD. When dilution was done to reduce the NH_4^+, other macro and micronutrient levels also reduced in DD resulting overall poor plant performances.

2.8. References

Adams P (2002) Nutritional control in hydroponics. In: Savvas, D., Passam, H.C. (Eds.), Hydroponic Production of Vegetables and Ornamentals. Embryo Publications, Athens, Greece,

Ahmad R, Jabeen N (2009) Demonstration of growth improvement in sunflower (helianthus annuus l.) By the use of organic fertilizers under saline conditions

Ahring B (2003) Preface. In: Ahring BK (ed) Biomethanation II. Springer, Berlin.

Akhavan HR, Barzegar M (2017) Determination of water-soluble vitamins in 15 Iranian pomegranate cultivars and their variation after pasteurization and cold storage. . Int Food Res J 24:1429

Alburquerque JA, de la Fuente C, Ferrer-Costa A, et al (2012) Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass and bioenergy,. Elsevier 40:181–189

Arnon DI (1949) Copper enzymes in isolated chloropolasts. polyphenoloxidase in (Beta vulgaris). Plant Physiol 1–15

Bachmann S, Uptmoor R, Eichler-Löbermann B (2016) Phosphorus distribution and availability in untreated and mechanically separated biogas digestates. Sci Agric 73:9–17

Bachmann S, Wentzel S, Eichler-Löbermann B (2011) Codigested dairy slurry as a phosphorus and nitrogen source for Zea mays L. and Amaranthus cruentus L. J Plant Nutr Soil Sci 174:908–915

Barker A V., Eaton TE, Meagy MJ, et al (2017) Variation of mineral nutrient contents of modern and heirloom cultivars of cabbage in different regimes of soil fertility. J Plant Nutr 40:2432–2439

Barker A V., Maynard DN, Lachman WH (1967) Induction of tomato stem and leaf lesions, and potassium deficiency, by excessive ammonium nutrition. Soil Sci 103:319–327

Barth C, Gouzd ZA, Steele HP, Imperio RM (2010) A mutation in GDP-mannose pyrophosphorylase causes conditional hypersensitivity to ammonium, resulting in Arabidopsis root growth inhibition, altered ammonium metabolism, and hormone homeost. J Exp Bot 61:379-394.

Barzee TJ, Edalati A, El-Mashad H, et al (2019) Digestate biofertilizers support similar or higher tomato yields and quality than mineral fertilizer in a subsurface drip fertigation system. Front Sustain Food Syst 3:58

Bauer A, Mayr H, Hopfner-Sixt K, Amon T (2009) Detailed monitoring of two biogas plants and mechanical solid–liquid separation of fermentation residues. J Biotechnol 142:56–63

Bauer R, Reminger P, Jurcic K, Wagner H (1989a) Influence of Echinacea extracts on phagocytic activity. Z Phytother 10:43–48

Bauer R, Reminger P, Jurcic K, Wagner H (1989b) Influence of Echinacea extracts on phagocytic activity. Z Phytother 10:43–48

Benbrook C, Zhao X, Yáñez J, et al (2008) New Evidence Confirms the Nutritional Superiority of Plant-Based Organic Foods. State of Science Review; The Organic Center: Boulder, CO, USA,

Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal Biochem 239:70–76

Bittsánszky A, Pilinszky K, Gyulai G, Komives T (2015) Overcoming ammonium toxicity. Plant Sci 231:184–190

Bloch D, Monshausen G, Singer M, et al (2011) Nitrogen source interacts with ROP signalling in

root hair tip-growth. Plant, Cell Environ 34:76–88

Bonin CP, Potter I, Vanzin GF, Reiter W-D (1997) The MUR1 gene of Arabidopsis thaliana encodes an isoform of GDP-D-mannose-4, 6-dehydratase, catalyzing the first step in the de novo synthesis of GDP-L-fucose. Proceedings of the National Ac. Plant Biol 94:2085–2090

Boonpangrak S, Lalitmanat S, Suwanwong Y, et al (2016) Analysis of Ascorbic Acid and Isoascorbic Acid in Orange and Guava Fruit Juices Distributed in Thailand by LC-IT-MS/MS. Food Anal Methods 9:1616–1626

Börjesson P, Berglund M (2007) Environmental systems analysis of biogas systems—Part II: The environmental impact of replacing various reference systems. Biomass and Bioenergy 31:326–344

Bottoms TG, Smith RF, Cahn MD, Hartz TK (2012) Nitrogen requirements and N status determination of lettuce. HortScience 12:1768–1774

British Nutrition F (2009) Minerals and trace elements. Assessed on: March 06, 2020. Available at: https://www.nutrition.org.uk/nutritionscience/nutrients-food-and-ingredients/minerals-and-trace-elements.html?showall=1&limitstart

Britto DT, Kronzucker HJ (2002) NH4+ toxicity in higher plants: a critical review. J Plant Physiol 159:567–584

Bryson GM, Mills HA, Sasseville DN, et al (2014) Plant analysis handbook III. Athens, GA: Micro-Macro Publishing

Buchanan D, Omaye S (2013) Comparative Study of Ascorbic Acid and Tocopherol Concentrations in Hydroponic- and Soil-Grown Lettuces. Food Nutr Sci 10:1047–1053.

Cano A, Acosta M, Arnao MB (2003) Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (Lycopersicon esculentum Mill.). Postharvest Biol

Technol 28:59–65

Carr AC, Vissers MCM (2013) Synthetic or food-derived vitamin C—are they equally bioavailable? Nutrients 5:4284–4304

Chantigny M, Angers D, Belanger G, et al (2008) Yield and nutrient export of grain corn fertilized with raw and treated liquid swine manure. Agron J 1303–1309. https://doi.org/10.2134/agronj2007.0361

Chantigny MH, Angers DA, Rochette P, et al (2007) Gaseous Nitrogen Emissions and Forage Nitrogen Uptake on Soils Fertilized with Raw and Treated Swine Manure. J Environ Qual 36:1864–1872

Chapman HD, Pratt PF (1962) Methods of analysis for soils, plants and waters. Soil Sci 93:68

Cometti NN, Bremenkamp DM, Galon K, et al (2013) Cooling and concentration of nutrient solution in hydroponic lettuce crop. Hortic Bras 31:287–292

Conklin PL, Norris SR, Wheeler GL, et al (1999a) Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis. Proc Natl Acad Sci 96:4198–4203

Conklin PL, Norris SR, Wheeler GL, et al (1999b) Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis

Coronel G, Chang M, Rodríguez-Delfín A (2008) Nitrate reductase activity and chlorophyll content in lettuce plants grown hydroponically and organically. In International Symposium on Soilless Culture and Hydroponics 843. pp 137–144

Correia ECS (2013) Reação de cultivares de alface do grupo americano a Meloidoygine incognita, M. javanica e M. enterolobii (55f. Dissertação (Mestrado em Agronomia), Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, Brazil).

Craig W, Beck L (1999) Phytochemicals: Health Protective Effects. Can J Diet Pract Res 60:78–

84

Cuevas-Rodri´guez EO, Rodri´guez R, Dia VP, et al (2010) Inhibition of Pro-inflammatory Responses and Antioxidant Capacity of Mexican Blackberry (Rubus spp.) Extracts. J Agric Food Chem 58:9542–9548. https://doi.org/10.1021/jf102590p

Dai J, Mumper RJ (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15:7313–7352

Davis JM, Murphy EA, Carmichael MD (2009) Effects of the dietary flavonoid quercetin upon performance and health. Curr Sport Med Rep 8:206–213

Dias JS, Ryder E (2011) World Vegetable Industry: Production, Breeding, Trends. Hort Rev 38:299–356

Drennan MF, DiStefano TD (2010) Characterization of the curing process from high-solids anaerobic digestion. Bioresour Technol 2:537–544

Duarte TL, Lunec J (2005) When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C. Free Radic Res 39:671–686

Durazzo A, Azzini E, Lazzé MC, et al (2014) Antioxidants in Italian head lettuce (lactuca sativa var. capitatal.) Grown in organic and conventional systems under greenhouse conditions. J Food Biochem 38:56–61

El-Shinawy MZ, Gawish SM (2006) Effect of commercial organic nutrient solutions on growth and chemical composition of lettuce under agricultural soilless system. Egypt J Hortic 33:19

Environmental Protection Agency (1979) Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020

Errebhi M, Wilcox GE (1990) Plant species response to ammonium-nitrate concentration ratios. J Plant Nutr 13:1017–1029

Esteban R, Ariz I, Cruz C, Moran J (2016) Review: Mechanisms of ammonium toxicity and the quest for tolerance. Plant Sci 248:92–101.

Fan J, Xiao J, Liu D, et al (2017) Effect of application of dairy manure, effluent and inorganic fertilizer on nitrogen leaching in clayey fluvo-aquic soil: A lysimeter study. Sci. Total Env 592:206–214

FAO (2018) The state of food insecurity in the world 2018. Available at: http://www.fao.org/state-of-food-security-nutrition. Assessed on: (April 20, 2020). Public Library of Science

Fernández-Cirelli A, Arumí JL, Rivera D, Boochs PW (2009) Environmental effects of irrigation in arid and semi-arid regions. Chil J Agric Res 27–40

Fishman M, Friedman L (1989) Methods for determination of inorganic substances in water and fluvial sediments. US Department of the Interior, Geological Survey.

Forge T, Kenney E, Hashimoto N, et al (2016) Compost and poultry manure as preplant soil amendments for red raspberry: Comparative effects on root lesion nematodes, soil quality and risk of nitrate leaching. Agric Ecosyst Environ 223:48–58

Fullbloom (2020) Hydroponic Systems 101 | Learn The Basics of Hydroponics. Available at: https://www.fullbloomgreenhouse.com/hydroponic-systems-101/ (Assessed on July 05, 2020).

Furukawa Y, Hasegawa H (2006) Response of Spinach and Komatsuna to Biogas Effluent Made from Source-Separated Kitchen Garbage. J Environ Qual 35:1939–1947

Gagliardini E, Buelli S, Benigni A (2011) Endothelin in chronic proteinuric kidney disease. Contrib Nephrol 172:171-184.

Garbin ML, Dillenburg LR (2008) Effects of different nitrogen sources on growth, chlorophyll

concentration, nitrate reductase activity and carbon and nitrogen distribution in Araucaria angustifolia. Brazilian J Plant Physiol 20:295–303

Gavrilova V, Kajd M, Gjamovski V, Stefova M (2011) Separation, Characterization and Quantification of Phenolic Compounds in Blueberries and Red and Black Currants by HPLCÀDADÀESI-MS n. J Agric Food Chem 59:4009–4018

Gell K, van Groenigen J, Cayuela ML (2011) Residues of bioenergy production chains as soil amendments: immediate and temporal phytotoxicity. J Hazard Mater 186:2017–2025

Georgacakis D, Sievers DM, Iannotti EL (1982) Buffer stability in manure digesters. Agric Wastes 4:427–441

Gerendás J, Zhu Z, Bendixen R, et al (1997) Physiological and biochemical processes related to ammonium toxicity in higher plants. J Plant Nutr Soil Sci 160:239–251

Gliszczyńska-Świgło A (2007) Folates as antioxidants. Food Chem 101:1480–1483

Goddek S, Schmautz Z, Scott B, et al (2016) The effect of anaerobic and aerobic fish sludge supernatant on hydroponic lettuce. Agronomy 2:37

Gómez X, Cuetos MJ, García AI, Morán A (2007) An evaluation of stability by thermogravimetric analysis of digestate obtained from different biowastes. J Hazard Mater 149:97–105

Gong W, Yan X, Wang J, et al (2011) Long-term applications of chemical and organic fertilizers on plant-available nitrogen pools and nitrogen management index. Biol Fertil Soils 47:767–775

Gupta UC, Gupta SC (2014) Sources and deficiency diseases of mineral nutrients in human health and nutrition: a review. Pedosphere 24:13–38

Hao XJ, Hong JP, Gao WJ (2007) Effect of biogas slurry and biogas residues on quality of

greenhouse mini cucumber. [J] Soil Fertil Sci China 5:9

Hariadi YC, Nurhayati AY, Hariyani P (2016) Biophysical monitoring on the effect on different composition of goat and cow manure on the growth response of maize to support sustainability. Agric Agric Sci Procedia 118–127

Harris E (2001) Copper homeostasis: the role of cellular transporters. Nutr Rev 59:281–285

Hassan SA, Mijin S, Yusoff UK, et al (2012) Nitrate, ascorbic acid, mineral and antioxidant activities of Cosmos caudatus in response to organic and mineral-based fertilizer rates. Molecules 17:7843–7853

Haukioja E, Ossipov V, Koricheva J, et al (1998) Biosynthetic origin of carbon-based secondary compounds: cause of variable responses of woody plants to fertilization? Chemoecology 8:133–139

Healy EF, Sanders J, King PJ, Edwards Robinson WJ (2009a) A docking study of L-chicoric acid with HIV-1 integrase. J Mol Graph Model 27:584–589

Healy EF, Sanders J, King PJ, Robinson Jr WE (2009b) A docking study of L-chicoric acid with HIV-1 integrase. J Mol Graph Model 27:584–589

Heimler D, Isolani L, Vignolini P, Romani A (2009) Polyphenol content and antiradical activity of Cichorium intybus L. from biodynamic and conventional farming. Food Chem 114:765–770

Herrera E, Jiménez R, Aruoma OI, et al (2009) Aspects of Antioxidant Foods and Supplements in Health and Disease. Nutr Rev 67:140–144

Hjorth M, Christensen K V., Christensen ML, Sommer SG (2010) Solid—liquid separation of animal slurry in theory and practice. A review. Agron Sustain Dev 30:153–180

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil.

Circular. California agricultural experiment station, 347(2nd edit).

Hobson P, Wheatley A (1992) Anaerobic digestion – modern theory and practice. Elsevier Appl Sci 269

Hokkaido (2003) Research report of environment, resources, and recycling in Hokkaido, Sapporo. Civil Engineering Institute of Hokkaido, Sapporo (in Japanese).

Hoque MM, Ajwa HA, Smith R (2007) Nitrite and ammonium toxicity on lettuce grown under hydroponics. Commun Soil Sci Plant Anal 39:207–216

Hord NG, Tang Y, Bryan NS (2009a) Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 90:1-10.

Hord NG, Tang Y, Bryan NS (2009b) Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 90:1–10

Hung HC, Joshipura KJ, Jiang R, et al (2004) Fruit and vegetable intake and risk of major chronic disease. J Natl Cancer Inst 96:1577–1584

Ibrahim MH, Jaafar HZ, Karimi E, Ghasemzadeh A (2013) Impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kacip Fatimah (Labisia pumila Benth). Molecules 18:10973–10988

Insam H, Gómez-Brandón M, Ascher J (2015) Manure-based biogas fermentation residues–Friend or foe of soil fertility? Soil Biol Biochem 84:1–14

IPL (2020) Hydroponics Advantages And Disadvantages. Available at: https://www.ipl.org/essay/Hydroponics-Advantages-And-Disadvantages-PKNBD2NFCEDR (Assessed on July 05, 2020).

Joshi R, Adhikari S, Patro BS, et al (2001) Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. Free Radic Biol Med 30:1390–1399

Kapoulas N, Koukounaras A, Ilić ZS (2017) Nutritional quality of lettuce and onion as companion plants from organic and conventional production in north Greece. ElsevierScientia Hortic 219:310–318

Karakaya S (2004) Bioavailability of Phenolic Compounds. Crit Rev Food Sci Nutr 44:453–464

Kempinski CF, Haffar R, Barth C (2011) Toward the mechanism of NH4+ sensitivity mediated by Arabidopsis GDP-mannose pyrophosphorylase. Plant, Cell Environ 34:847–858

Kim MJ, Moon Y, Tou JC, et al (2016) Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). J Food Compos Anal 49:19–34

Kirchmann H, Bernal M (1997) Organic waste treatment and C stabilization efficiency. Soil Biol Biochem 29:1747–1753

Klouberta V, Rink. L (2015) Zinc as a micronutrient and its preventive role of oxidative damage in cells. Food Funct 6:3195–3204

Knapp Haraldsen T, Andersen U, Krogstad T, Sørheim R (2011) Liquid digestate from anaerobic treatment of source-separated household waste as fertilizer to barley. Waste Manag Res 29:1271–1276

Koester RP, Skoneczka JA, Cary TR, et al (2014) Historical gains in soybean (Glycine max Merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. J Exp Bot 12:3311–3321

Kris-Etherton PM, Hecker KD, Bonanome A, et al (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 9:71–88

Krupa S V. (2003) Effects of atmospheric ammonia (NH3) on terrestrial vegetation: a review. Envionmental Pollut 124:179–221

Lafleur B, Thiffault E, Paré D, et al (2012) Effects of hog manure application on the nutrition

and growth of hybrid poplar (Populus spp.) and on soil solution chemistry in short-rotation woody crops. Agric Ecosyst environmen 155:95–104

Lam V, Watanabe T, Phan T, Khai L (2002) A case study: introduction of low-cost biogas digester to small-scale farming systems

Lampkin N (1990) Organic farming. Farming press books. Ipswich. United Kingdom.

Li B, Li G, Kronzucker HJ, et al (2014) Ammonium stress in Arabidopsis: signaling, genetic loci, and physiological targets. Trends Plant Sci 19:107–114

Li B, Li Q, Xiong L, et al (2012) Arabidopsis plastid AMOS1/EGY1 integrates abscisic acid signaling to regulate global gene expression response to ammonium stress. Plant Physiol 160:2040–2051

Li BH, Shi. WM (2007) Effects of elevated NH4+ on Arabidopsis seedlings different in accessions. Acta Pedofil Sin 44:508–515

Li Q, Li X, Tang B, Gu M (2018) Growth responses and root characteristics of lettuce grown in aeroponics, hydroponics, and substrate culture. Horticulturae 4:35

Liedl BE, Cummins M, Young A, et al (2004) Hydroponic lettuce production using liquid effluent from poultry waste bioremediation as a nutrient source. In VII International Symposium on Protected Cultivation in Mild Winter Climates: Production, Pest Management and Global Competition 659. pp 721–728

Liedl BE, Wilfong K, Taylor C, Mazzaferro K (2006) Liquid effluent from poultry waste bioremediation as a nutrient source for hydroponic cucumber production. HortScience 4:997A – 997

Lin D, Xiao M, Zhao J, et al (2016) An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules 21:1374

Lind OP, Hultberg M, Bergstrand KJ, et al (2020) Biogas Digestate in Vegetable Hydroponic Production: pH Dynamics and pH Management by Controlled Nitrification. Waste and Biomass Valorization 1–11

Liu WK, Yang QC, Du L (2009) Soilless cultivation for high-quality vegetables with biogas manure in China: Feasibility and benefit analysis. Renewable Agriculture and Food Systems,. Renew Agric Food Syst 300–307

Liu WK, Yang QC, du Lian F, et al (2011) Nutrient supplementation increased growth and nitrate concentration of lettuce cultivated hydroponically with biogas slurry. Acta Agric Scand Sect B Soil Plant Sci 61:391–394

Liu X, Ardo S, Bunning M, et al (2007) Total phenolic content and DPPH radical scavenging activity of lettuce (Lactuca sativa L.) grown in Colorado. LWT Food Sci Technol 40:552–557

Liu Y, Lai N, Gao K, et al (2013) Ammonium inhibits primary root growth by reducing the length of meristem and elongation zone and decreasing elemental expansion rate in the root apex in Arabidopsis thaliana. PLoS One 8:e61031.

Liu YJ, Tong YP, Zhu YG, et al (2006) Leaf chlorophyll readings as an indicator for spinach yield and nutritional quality with different nitrogen fertilizer applications. J Plant Nutr 29:1207–1217

Llorach R, Martínez-Sánchez A, Tomás-Barberán FA, et al (2008) Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem 108:1028–1038

Loqué D, Mora SI, Andrade SL, et al (2009) Pore mutations in ammonium transporter AMT1 with increased electrogenic ammonium transport activity. J Biol Chem 284:24988–24995

Loria ER, Sawyer JE, Barker DW, et al (2007) Use of anaerobically digested swine manure as a nitrogen source in corn production. In: Agronomy Journal. pp 1119–1129

Madeira AC, De Varennes A (2005) Use of chlorophyll meter to assess the effect of nitrogen on sweet pepper development and growth. J Plant Nutr 28:1133–1144

Makádi M, Orosz A, Tomócsik V (2012) Digestate: A New Nutrient Source - Review, Biogas, Dr. Sunil Kumar (Ed.), ISBN: 978-953-51-0204-5, InTech, Available from: http://www.intechopen.com/books/biogas/digestate-a-new-nutrient-source-review

Makádi M, Tomócsik A, Kátai J, et al (2008) Nutrient cycling by using residues of bioenergy production - effects of biogas-digestate on plant and soil parameters. Cereal Res Commun 1807–1810

Mampholo BM, Maboko MM, Soundy P, Sivakumar D (2016) Phytochemicals and Overall Quality of Leafy Lettuce (Lactuca sativa L.) Varieties Grown in Closed Hydroponic System. J Food Qual 39:805–815

Man J, Shi Y, Yu Z, Zhang Y (2015) Dry matter production, photosynthesis of flag leaves and water use in winter wheat are affected by supplemental irrigation in the Huang-Huai-Hai Plain of China. PLoS One 10:e0137274.

Mapson LW, Cruickshank EM (1947) Effect of various salts on the synthesis of ascorbic acid and carotene in cress seedlings. Biochem J 41:197-205.

Masarirambi MT, Hlawe MM, Oseni OT, Sibiya TE (2010) Effects of organic fertilizers on growth, yield, quality and sensory evaluation of red lettuce (Lactuca sativa L.) ‘Veneza Roxa.’ Agric Biol J N Am, 1:1319–1324

Massé DI, Talbot G, Gilbert Y (2011) On farm biogas production: A method to reduce GHG emissions and develop more sustainable livestock operations. Anim Feed Sci Technol 436–

445

Mayer-Foulkes DA (2011) A survey of macro damages from non-communicable chronic diseases: Another challenge for global governance. Glob Econ J 11:1–26

Meagy MJ, Eaton TE, Barker A V. (2016) Assessment of mineral nutrient concentrations of lettuce in response to cultivar selection and fertilization in greenhouse production. J Plant Nutr 39:1796–1808

Medicine I of (2005) Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Institute of Medicine, Washington D.C. National Academies Press (US).

Medicine I of (1997) Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Institute of Medicine, Washington, D.C. National Academies Press (US).

Medicine I of (1998) Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Institute of Medicine, Washington, D.C. National Academies Press (US).

Medina E (1984) Nutrient balance and physiological processes at the leaf level. In Physiological ecology of plants of the wet tropics (pp. 139-154). Springer, Dordrecht. pp 139–154

Menardo S, Gioelli F, Balsari P (2011) The methane yield of digestate: effect of organic loading rate, hydraulic retention time, and plant feeding. Bioresour Technol 102:2348–2351

Mihaljević S, Radić S, Bauer N, et al (2011) Ammonium-related metabolic changes affect somatic embryogenesis in pumpkin (Cucurbita pepo L.). J Plant Physiol 168:1943–1951

Miller J, Rucker R (2012) Pantothenic acid. In: Erdman JW, Macdonald IA, Zeisel SH, eds. Present Knowledge in Nutrition. 10th ed. Washington, DC: Wiley-Blackwell:375-90.

Mohankumar JB, Uthira L, Maheswari SU (2018) Total phenolic content of organic and conventional green leafy vegetables. J Nutr Hum Heal 2:1–6

Mollavali M, Perner H, Rohn S, et al (2018) Nitrogen form and mycorrhizal inoculation amount and timing affect flavonol biosynthesis in onion (Allium cepa L.). Mycorrhiza 28:59–70

Möller K, Schulz R, Müller T (2010) Substrate inputs, nutrient flows and nitrogen loss of two centralized biogas plants in southern Germany. Nutr Cycl Agroecosystems 87:307–325

Möller K, Stinner W, Deuker A, Leithold G (2008a) Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. Nutr Cycl Agroecosystems 82:209–232

Möller K, Stinner W, Deuker A, Leithold G (2008b) Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. Nutr Cycl Agroecosystems 82:209–232

Morris MC, Evans DA, Tangney CC, et al (2006) Associations of vegetable and fruit consumption with age-related cognitive change. Neurology 67:1370–1376

Mou B (2009) Nutrient content of lettuce and its improvement. Curr Nutr Food Sci 5:242-248.

Mou B, Ryder EJ (2004) Relationship between the nutritional value and the head structure of lettuce. Acta Hortic 637:361–7

Mulla D, Birr A, Randall G, et al (2001) Impacts of animal agriculture on water quality: technical work paper. Minnesota Environmental Quality Board, Minnesota Planning (Agency), St. Paul.

Nicola S, Hoeberechts J, Fontana E (2005) Comparison between traditional and soilless culture systems to produce rocket (Eruca sativa) with low nitrate content. In International Symposium on Soilless Culture and Hydroponics; International Society for. pp 549–555.

Nicoletto C, Santagata S, Zanin G, Sambo P (2014) Effect of the anaerobic digestion residues use on lettuce yield and quality. Sci Hortic (Amsterdam) 180:207–213

Nicolle C, Carnat A, Fraisse D, et al (2004) Characterisation and variation of antioxidant micronutrients in lettuce (Lactuca sativa folium). J Sci Food Agric 84:2061–2069

Nkoa R (2014) Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. Agron Sustain Dev 34:473-492.

Norenberg MD, Rama Rao K V., Jayakumar AR (2009) Signaling factors in the mechanism of ammonia neurotoxicity. Metab. Brain Dis. 24:103–117

Olsen KM, Lea US, Slimestad R, et al (2008) Differential expression of four Arabidopsis PAL genes; PAL1 and PAL2 have functional specialization in abiotic environmental-triggered flavonoid synthesis. J Plant Physiol 165:1491–1499

Paavola T, Rintala J (2008) Effects of storage on characteristics and hygienic quality of digestates from four co-digestion concepts of manure and biowaste. Bioresour Technol 99:7041–7050

Pan Z, Qi G, Andriamanohiarisoamanana FJ, et al (2018) Potential of anaerobic digestate of dairy manure in suppressing soil-borne plant disease. Anim Sci J 89:1512–1518

Pavia M, Pileggi C, Nobile CGA, Angelillo IF (2006) Association between fruit and vegetable consumption and oral cancer: a meta-analysis of observational studies. Am J Clin Nutr 83:1126–1134

Pellati F, Benvenuti S, Magro L, et al (2004) Analysis of phenolic compounds and radical scavenging activity of Echinacea spp. J Pharm Biomed Anal 35:289–301

Pérez-Jiménez J, Neveu V, Vos F, Scalbert A (2010) Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. Eur J Clin Nutr 64:112–120

Petropoulos SA, Chatzieustratiou E, Constantopoulou E, Kapotis G (2016) Yield and quality of

lettuce and rocket grown in floating culture system. Not Bot Horti Agrobot Cluj-Napoca 44:603–612

Pognani M, D'Imporzano G, Scaglia B, Adani F (2009) Substituting energy crops with organic fraction of municipal solid waste for biogas production at farm level: A full-scale plant study. Process Biochem 44:817–821

Przygocka-Cyna K, Grzebisz W, Lukowiak R, Biber M (2018) Biomass ash and biogas digestate bio-fertilizers as a source of nutrients for light acid soil–an exhaustion test. J Elem 23:413–428

Qi X, Zhang S, Wang Y, Wang R (2005) Advantages of the integrated pig-biogas-vegetable greenhouse system in North China. Ecol Eng 24:175–183

Qin C, Qian W, Wang W, et al (2008) GDP-mannose pyrophosphorylase is a genetic determinant of ammonium sensitivity in Arabidopsis thaliana

Qu H, Madl RL, Takemoto DJ, et al (2005) Lignans are involved in the antitumor activity of wheat bran in colon cancer SW480 cells. J Nutr 3:598–602

Rajashekar CB, Oh M-M, Carey EE (2012) Organic Crop Management Enhances Chicoric Acid Content in Lettuce. Food Nutr Sci 3:1296–1302

Rezk BM, Haenen GR, van der Vijgh WJ, Bast A (2003) Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophores. FEBS Letters. Elsevier 555:601– 605

Roberts S (2018) What Is the Main Function of Phosphorus in the Body? Assessed on: March 06, 2020. Available at: https://healthyeating.sfgate.com/main-function-phosphorus-body-5789.html

Ronga D, Setti L, Salvarani C, et al (2019) Effects of solid and liquid digestate for hydroponic

baby leaf lettuce (Lactuca sativa L.) cultivation. Sci Hortic (Amsterdam) 172–181

Roosta HR, Schjoerring JK (2007) Effects of ammonium toxicity on nitrogen metabolism and elemental profile of cucumber plants. J Plant Nutr 30:1933–1951

Sacks FM, Willett WC, Smith A, et al (1998) Effect on Blood Pressure of Potassium, Calcium, and Magnesium in Women With Low Habitual Intake. Hypertension 31:131–138

Salminen E, Rintala J, Härkönen J, et al (2001) Anaerobically digested poultry slaughterhouse wastes as fertiliser in agriculture. Bioresour Technol 78:81–88

Santamaria P (2006) Nitrate in vegetables: Toxicity, content, intake and EC regulation. J. Sci. Food Agric. 86:10–17

Santos FT, Goufo P, Santos C, et al (2016) Comparison of five agro-industrial waste-based composts as growing media for lettuce: Effect on yield, phenolic compounds and vitamin C. Food Chem 209:293–301

Sapkota S, Sapkota S, Liu Z (2019) Effects of nutrient composition and lettuce cultivar on crop production in hydroponic culture. Horticulturae 5:72

Savvas D, Passam HC, Olympios C, et al (2006) Effects of Ammonium Nitrogen on Lettuce Grown on Pumice in a Closed Hydroponic System

Scaglia B, Pognani M, Adani F (2017) The anaerobic digestion process capability to produce biostimulant: the case study of the dissolved organic matter (DOM) vs. auxin-like property. Sci Total Environ 589:36-45.

Scott J, Rébeillé F, Fletcher J (2000) Folic acid and folates: The feasibility for nutritional enhancement in plant foods. J. Sci. Food Agric. 80:795–824

Seefeldt S (2015) Crop Production and Soil Mangement Series Steven Seefeldt, Extension Faculty, Agriculture and Horticulture. Assessed on: September 14, 2020. Available at:

http://cespubs.uaf.edu/index.php/download_file/1025/.

Shohag MJI, Wei Y, Yang X (2012) Changes of Folate and Other Potential Health-Promoting Phytochemicals in Legume Seeds As Affected by Germination. J Agric Food Chem 60:9137–9143

Silber A, Bar-Tal A (2008) Nutrition of substrate-grown plants. Soil Cult Theory Pract Elsevier, San Diego, CA, 4:291-339.

Slamet W, Purbajanti ED, Darmawati A, Fuskhah E (2017) Leaf area index, chlorophyll, photosynthesis rate of lettuce (Lactuca sativa L) under N-organic fertilizer. Indian J Agric Res 4:365–369

Soetan KO, Olaiya CO, Oyewole OE (2010) The importance of mineral elements for humans, domestic animals and plants: A review. African J food Sci 5:200-222.

Somasundaram E, Amanullah MM, Vaiyapuri K, et al (2007) Influence of organic sources of nutrients on the yield and economics of crops under maize based cropping system. J Appl Sci Res 1774-1777.

Sommer SG, Husted S (1995a) A simple model of pH in slurry. J Agric Sci 124:447–453

Sommer SG, Husted S (1995b) The chemical buffer system in raw and digested animal slurry

Southon S (2000) “Increased Fruit and Vegetable Consumption within the EU: Potential Health Benefits,” Food Research International. 33:211-217.

Squire GR, Ong CK, Monteith JL (1987) Crop growth in semi-arid environment. In: Proceedings of 7th International Workshop, 7-11 April, 1986, International Crops Research Institute for Semi-Arid Tropics. Patancheru, Hyderabad, pp 219-231.

Stinner W, Möller K, Leithold G (2008) Effects of biogas digestion of clover/grass-leys, cover crops and crop residues on nitrogen cycle and crop yield in organic stockless farming

systems. Eur J Agron 29:125–134

Stoknes K, Scholwin F, Krzesiński W, et al (2016) Efficiency of a novel "Food to waste to food" system including anaerobic digestion of food waste and cultivation of vegetables on digestate in a bubble-insulated greenhouse. Waste Manag 466–476

Su YY, Lu Y, Shi WS (2008) Effect of biogas fertilizer on yield and quality of lettuce in soilless culture. [J] Soil Fertil Sci China 1

Sulaiman SF, Yusoff NAM, Eldeen IM, et al (2011) Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (Musa sp.). J Food Compos Anal 1–10.

Survase SA, Bajaj IB, Singhal RS (2006) Biotechnological production of vitamins. Food Technol Biotechnol 44:381–396

Szűcs B, Simon M, Füleky G (2006) Anaerobic pre-treatment effects on the aerobic degradability of wastewater sludge. Proceedings of the Internationale Conference ORBIT 2006, Weimar, 13-15 September, 2006. Part 2, pp. pp 425-434.

Tambone F, Genevini P, D'Imporzano G, Adani F (2009) Assessing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW. Bioresour Technol 100:3140–3142

Tambone F, Scaglia B, D'Imporzano G, et al (2010) Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 5:577–583

Tamilselvi SM, Chinnadurai C, Ilamurugu K, et al (2015) Effect of long-term nutrient managements on biological and biochemical properties of semi-arid tropical Alfisol during

maize crop development stages. Ecol Indic 48:76–87

Tani M, Sakamoto N, Kishomoto T, Umetsu K (2006) Utilization of anaerobically digested slurry combined with other waste following application to agricultural land. Int Congr Ser 1293:331.

Teglia C, Tremier A, Martel JL (2011) Characterization of solid digestates: Part 2, assessment of the quality and suitability for composting of six digested products. Waste and Biomass Valorization 2:113–126

Tian X, Li S, Wang Z, et al (2003) Response of lettuce to different nitrogen forms. Ying yong sheng tai xue bao=. J Appl Ecol 14:377

Tiquia SM, Tam NFY, Hodgkiss IJ (1996) Effects of composting on phytotoxicity of spent pig-manure sawdust litter. Environ Pollut 93:249–256

Tiwari VN, Tiwari KN, Upadhyay RM (2000) Effect of crop residues and biogas slurry incorporation in wheat on yield and soil fertility. J Indian Soc Soil Sci 48:515–520

Trumbo P (2014) Pantothenic acid. In: Ross AC, Caballero B, Cousins RJ, et al., eds. Modern Nutrition in Health and Disease. 11th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2014:351-7.

Tshikalange B, Bello ZA, Ololade OO (2020) Comparative nutrient leaching capability of cattle dung biogas digestate and inorganic fertilizer under spinach cropping condition. Environ Sci Pollut Res 27:3237–3246

USDA (2015) National Nutrient Database for Standard Reference Release 28. USDA, Washington, D.C.

Valverde-Barrantes OJ, Freschet GT, Roumet C, Blackwood CB (2017) A worldview of root traits: the influence of ancestry, growth form, climate and mycorrhizal association on the

functional trait variation of fine-root tissues in seed plants. New Phytol 215:1562–1573

Wargovich MJ (2000) Anticancer Properties of Fruits and Vegetables

Warman PR, Havard KA (1997) Yield, vitamin and mineral contents of organically and conventionally grown carrots and cabbage. Agric Ecosyst Environ 61:155–162

Watson SA, Noggle. GR (1947) Effect of mineral deficiencies upon the synthesis of riboflavin and ascorbic acid by the oat plant. Plant Physiol 22:228–243

Webb AR, Hawkes FR (1985) Laboratory scale anaerobic digestion of poultry litter: Gas yield-loading rate relationships. Agric wastes 13:31–49

Weiland P (2010) Biogas production: current state and perspectives. Appl Microbiol Biotechnol 849–860

Wenke L, Du L, Yang Q (2009) Biogas slurry added amino acids decreased nitrate concentrations of lettuce in sand culture. Acta Agric Scand Sect B Soil Plant Sci 59:260–264

Wright AF, Bailey JS (2001) Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 dry combustion analyzer. Commun Soil Sci Plant Anal 32:3243–3258

Xu WH, Wang ZY, Quan YM, et al (2003a) Effect of application of biogas slurry on nitrate content and nutrition quality of lettuce and romaine lettuce. Rural Eco-Environment, 19:34–37

Xu WH, Wang ZY, Wang Q, et al (2003b) Effects of different biogas slurry on nutrient quality and nitrate content of lettuce. China biogas 21:11–13

Yalovsky S, Bloch D, Sorek N, Kost B (2008) Regulation of membrane trafficking, cytoskeleton dynamics, and cell polarity by ROP/RAC GTPases. Plant Physiol 147:1527–1543

You Q, Chen F, Wang X, et al (2012) Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. LWT-Food Sci Technol 46:164–168

Yu FB, Luo XP, Song CF, et al (2010) Concentrated biogas slurry enhanced soil fertility and tomato quality. Acta Agric Scand Sect B Soil Plant Sci 60:262–268

Zandvakili OR, Barker A V., Hashemi M, et al (2019) Comparisons of commercial organic and chemical fertilizer solutions on growth and composition of lettuce. J Plant Nutr 42:990–1000

Zandvakili OR, Ebrahimi E, Hashemi M, et al (2017) The potential of green manure mixtures to provide nutrients to a subsequent lettuce crop. Commun Soil Sci Plant Anal 48:2246–2255

Zhao X, Carey EE, Young JE, et al (2007) Influences of organic fertilization, high tunnel environment, and postharvest storage on phenolic compounds in lettuce. HortScience 42:71–76

Zuo J, Tang W, Xu Y (2015) Anti-Hepatitis B Virus Activity of Chlorogenic Acid and Its Related Compounds. In Coffee in health and disease prevention (pp. 607-613). Academic Press.

Chapter 3

3. Influence of dairy digestate on phytochemical profile of hydroponically grown lettuce in controlled environment

3.1. Abstract

Lettuce (*Lactuca sativa* L.) contains an immense variety of mineral, vitamins and health promoting secondary metabolite which are beneficial and essential for good human health. It has been reported that organic fertilizer enhance secondary metabolites or phytochemical profile in vegetables compared to inorganic fertilizers. A pilot scale study was conducted to determine the qualitative traits of lettuce grown in DD in hydroponic system. Experimental treatments were two lettuce varieties: i) Newham, ii) Romaine, and three nutrient solutions: i) NS), ii) DD, and iii) 50% NS + 50% DD. The experiment was laid out in a completely randomized design with factorial arrangements and replicated three times. The results indicated that DD significantly affected minerals, vitamins and secondary metabolites concentration in lettuce cultivars. DD showed lower concentration of minerals and vitamin C and higher concentration of secondary metabolites such as chicoric acid, chlorogenic acid, luteoline, quercitin-3-b-d-gluconide, quercitin-3-glucoside and quercitin-b-malonyl in lettuce compared to NS. On the other hand, Romaine variety exhibited higher quality indices than Newham which could be due to genetic potential of cultivars. As DD enhanced phytochemical profile of lettuce, we suggest that DD can be used as nutrient source or organic fertilizer to increase secondary metabolites in lettuce cultivated in hydroponics, which can substantially contributes in improving human health.

Keywords:

Dairy digestate, nutrient solution, lettuce, minerals, vitamins, secondary metabolites, ammonium, hydroponics, human health

3.2. Introduction

Green leafy vegetables are a major source of micronutrients, provide more vitamins than any other food and therefore play a vital role in human nutrition due to their health benefits (Mohankumar et al. 2018). These vegetables are rich and chief source of various minerals and phytochemicals such as calcium (Ca), iron (Fe), vitamin C, folates, β-carotene, dietary fibers and many more trace minerals. Lettuce is one of the most popular green leafy vegetable consumed as a salad and considered as a good source of various health promoting compounds and phytochemicals such as phenolics, vitamin C, folates, and carotenoids (Nicolle et al. 2004). These phytochemicals possess ideal structural properties to scavenge the free radicals thus decreasing the oxidative stress (Sulaiman et al. 2011), which help in reducing the chronic diseases, modify the metabolic activation, detoxification of various carcinogens, or even manipulating the various progressions that help to amend the course of tumor cells (Southon 2000; Herrera et al. 2009). Vitamin C is such a non-enzymatic antioxidant which is involved in various biochemical mechanisms and is helpful in reducing the oxidative stress both in-vivo and in-vitro conditions (Duarte and Lunec 2005). Several studies suggested that folates may act as antioxidants (Joshi et al. 2001; Rezk et al. 2003) due to their comparable activity value to vitamins C and E (Gliszczyńska-Świgło 2007).

Composition, sources and management of fertilizer application may alter qualitative indices or phytochemical profile of leafy vegetables (Ibrahim et al. 2013). Various studies investigating organic and inorganic fertilizers on enhancing growth, yield and quality of vegetables in soil based system are well documented (Qi et al. 2005; Yu et al. 2010). DD is a byproduct of anaerobic digestion and consists of partially degraded organic matter, microbial biomass and various inorganic compounds which makes it a valuable bio-fertilizerHowever, effects of DD on the minerals, vitamins and phenolic compounds in hydroponically grown lettuce need to be investigated(Möller et al. 2008a; Alburquerque et al. 2012).

El-Shinawy and Gawish (2006) evaluated the effects of commercial organic and inorganic solutions on mineral composition of lettuce in soilless cultivation during two growing seasons. The results indicated that lettuce grown in inorganic solution showed higher concentration of P, potassium (K), Ca and magnisum (Mg), whereas, lettuce grown in organic solution showed higher total nitrogen. In another study conducted by Zandvakili et al. (2019) investigated the effects of organic solution i.e. (2% total N (1% water-soluble and 1% water-insoluble) with 1% available phosphate (P_2O_5; 0.44% P) and 1% soluble potash (K_2O; 0.83% K) and was applied at equivalent N as with Hoagland and Arnon solution) and chemical fertilizer (urea) on mineral composition and crude protein of lettuce in hydroponic system. They observed that lettuce grown in organic solution showed lower concentration of minerals and crude protein compared to inorganic fertilizers application. Meagy et al. (2016) conducted the experiment to assess the effect of three mineral nutrient i.e. Hoagland solution, organic fertilizer and a chemical fertilizer (20-4.4-16.6 NPK) on nine cultivars of lettuce in a greenhouse production system. They observed higher Ca, Mg, sulfur (S), zinc (Zn), boron (B) and iron (Fe) concentration in lettuce grown in inorganic fertilizers, whereas, P, K, Mn and Cu were higher in organic solution. They also observed that heritage cultivars had about 10% higher concentration of P, K, Ca, Mg and Zn than modern cultivars. Liedl et al. (2004) evaluated the effects of commercial inorganic fertilizer and poultry liquid waste on lettuce minerals in hydroponic system. They reported lower minerals concentration in lettuce grown in poultry liquid waste compared to commercial inorganic fertilizer. However, poultry liquid waste or organic fertilizer fed plants produced higher P, sulphur (S) and Zn than inorganic fertilizer. In another study, Liu et al. (2011) investigated the effect of biogas slurry in combination with different nutrient supplementation on vitamin C contents of hydroponically grown lettuce. They reported that lettuce grown in biogas slurry in combination with ethylenediaminetetraacetic acid (EDTA-Fe; iron chelate) showed higher concentration of vitamin C as compared to other biogas treatments. Similarly, Ibrahim et al. (2013) investigated the effects of inorganic fertilizer (15: 15: 15 NPK) and chicken dung (10% N:10% P_2O_5:10% K_2O) under different N rates i.e. 0, 90, 180 and 270 kg N/ha on phenolic contents of hydroponically grown Kacip Fatimah (*Labisia pumila* Benth). Chicken dung fertilizer enhanced total phenolics, flavonoids and ascorbic acid than inorganic fertilizer. Literature doesn't report much on the utilization of DD as a sole nutrient source in hydroponics. For example, in a recent study conducted by Lind et al. (2020) investigated the effects of DD on the growth and yield of lettuce. However, effects of DD alone and combination of DD with inorganic fertilizers on qualitative profile of lettuce has not been fully explored yet. Hence, we hypothesized that DD as organic nutrient source will enhance the minerals, vitamins and phytochemical profile of hydroponically grown lettuce under controlled environment. To test this hypothesis, we conducted two set of greenhouse experiments in hydroponics with following objectives:

I. to determine the effect of DD, NS and combination of NS+DD on minerals and vitamins in lettuce.

II. to investigate the effects of DD, NS alone and combination of both nutrient solutions on bioactive compounds (phenolic content, flavonoids, total antioxidants) in lettuce.

3.3. Materials and methods

3.3.1. Nursery raising, and plant growing conditions

The seeds of two lettuce varieties *viz.* Newham and Romaine were purchased from High Mowing Organic Seeds (Wolcott, VT, USA). The seeds were sown in Jiffy-7 pre-soaked peat pellets (Jeffy-7, Lorain, OH, USA) and were placed in a growth chamber (Biochambers, MB, Canada), located at Recplex, Grenfell Campus, Memorial University of Newfoundland, Canada. The growth chamber was equipped with a climate control system; growth conditions were 14/10 h day/night duration with 21 /19 °C temperature, 80% relative humidity, and 400 – 600 ppm CO_2. One-week old lettuce nursery plants were transplanted in a greenhouse located at St. David's (51.8812° N, 5.2660° W), Newfoundland and Labrador (NL), Canada. Photoperiod, temperature and relative humidity (RH) were 14/10 h day/night, 25/17 °C, and 75 - 80 % during both crop cycles. The Hobo meter (MX1102A, USA) was installed in the GH to continuously monitor the growth conditions throughout the crop cycles. The experimental treatments were three nutrient solution (modified Hoagland solution designated as NS, DD 100% and their combination (50 % NS+ 50% DD), and two lettuce varieties (Newham and Romaine). The experimental design was a completely randomized with factorial arrangement and replicated thrice.

3.3.2. Preparation of nutrient solutions

NS was prepared following the method of Hoagland and Arnon (1950). For DD solution, DD was sourced from NWD's anaerobic digester, a composite DD sample was sent to Agriculture and Food Laboratory, University of Guelph for complete nutrient and heavy metal analysis. The DD was sieved using 2 mm filter to remove the large particles or any other inert matter (Figure 8). The DD nutrient solution was prepared following DD analyses reports, particularly NH_4^+-N concentration was considered baseline for preparation of DD solution (Table 4). The analysis report showed very high NH_4^+-N concentration (2200 mg L^{-1}, Table 3) in DD. Therefore, DD was diluted 10 times to reduce NH_4^+-N concentration to attain required level. DD solution was prepared in 1000 L opaque plastic container. Combination of NS and DD solution (50% NS + 50% DD) was prepared considering 50 % N from DD and 50% N from NS (Table 4) and pH of all nutrient solutions was maintained between 5.8- 6.2.

Table 4: Physicochemical characterization of three nutrient feed solutions used in this study

	NS			NS+DD			DD		
	Report 1	Report 2	Report 3	Report 1	Report 2	Report 3	Report 1	Report 2	Report 3
pH	5.9	6	5.9	6	5.9	6	6	6.1	6.1
EC (mS m^{-1})	2.47	2.51	2.50	3.7	3.65	3.73	4.1	4.2	4.2
					(mg L^{-1})				
NO_3^-N	196	212	193	72	207	40.3	16.2	149	1.47
NH_4^+-N	8.64	8.24	9.61	122	142	191	292	300	396
P	52	56	59	26	27	34	91	46	54
K	370	384	390	190	270	280	250	230	250
Ca	225	220	230	110	190	170	220	110	130
S	98	101	110	24	34	41	35	27	34
Mg	47	51	48	28	26	25	92	45	42
Na	42	56	53	80	88	100	140	120	150
Fe	5.7	5.6	5.8	2.9	4.2	5.8	8.2	4.1	5.7
Cu	0.1	0.1	0.1	0.63	0.31	0.73	2.9	1.4	1.5
Mn	0.98	1.7	1.2	0.67	0.87	1	2.3	0.82	0.95
Zn	0.5	0.5	0.59	0.85	0.81	1.6	2.7	1.7	2.6

The feed solutions were analyzed on July 04 (7 days after transplanting (DAT)), August 28 (24 DAT), and October 03 (40 DAT) during 2019 and designated as Report 1, 2 and 3, respectively.

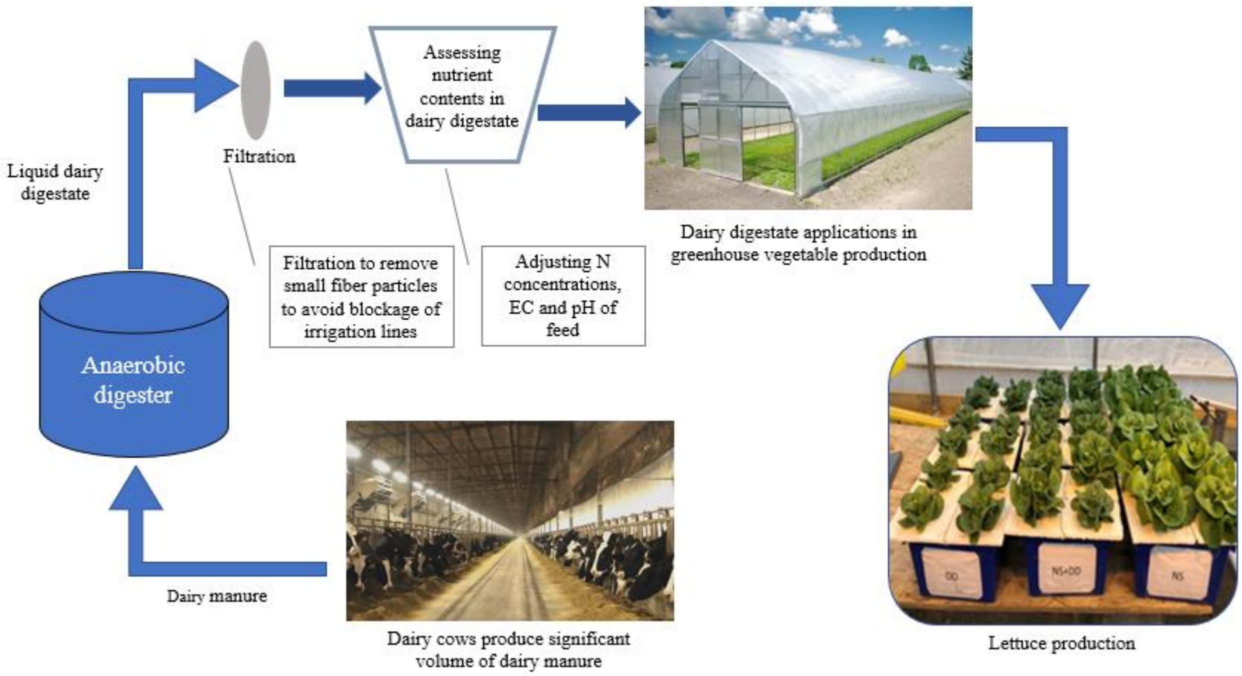

Figure 8: Flow chart showing dairy digestate collection, filtration, adjustment of nutrients and applications to hydroponically grown lettuce in a greenhouse.

3.3.3. Plant tissues analyses

3.3.4. Minerals analysis

Minerals content in lettuce leaves was determined following the method of Chapman and Pratt (1962). Briefly, 5 g of dried grounded lettuce leaf samples were weighed into a crucible, placed in muffle furnace for 5 h at 530 °C to make it ash, then ash was dissolved in 1 m L of hydrochloric acid (HCl) and then 25 mL distilled water was added. The resultant mixture was allowed to settle for about 30 min, and filtered through Whatman No. 42 filter paper. Thereafter, the aliquot was analyzed for elemental analyses using ICP-OES 725 (Inductively coupled plasma atomic emission spectroscopy) (Agilent Technologies; CA, USA).

3.3.5. Crude protein

Total nitrogen in lettuce leaves was determined following the method developed by Wright and Bailey (2001). Briefly, 5 g dried lettuce leaves were grounded to a fine powder with Cryo Mill (Retsch, Germany), then weighed into a crucible, placed in muffle furnace at 530 °C or 5 hours to convert into ash. Then ash samples (4.5±0.5 mg) were weighed in tin capsules and analyzed with CHNS combustion Analyzer (LECO model 928; St. Joseph, Michigan, USA) for total nitrogen contents. Crude protein contents were determined by multiplying the total nitrogen concentration by a universal conversion factor 6.25 as calculated by Zandvakili et al. (2019).

3.3.6. Vitamins

Leaf samples were prepared according to the methods reported by Boonpangrak et al. (2016) with minor modifications. Briefly, 5 g thawed lettuce leaves were homogenized with diatomaceous earth (~4 g) using mortar and pestle. Then mixture was loaded into 10 mL stainless steel Accelerated Solvent Extractor (ASE) cell and extracted on Dionex 350 ASE (Thermo Scientific, Massachusetts, USA) using methanol:water (60:40 % v/v) as extraction solvent based on the following program: (temperature = 0 °C; number of cycles = 2; pressure = 1500 psi; static time = 5 min; flush ratio = 50% rinse). The extracted analytes were stored in amber colored vials at -20 °C freezer for further analysis.

3.3.7. Vitamin C

Vitamin C content in lettuce leaves was measured based on ascorbic acid content following the method developed by Boonpangrak et al. (2016) with minor modifications. Briefly, solvent A was prepared by mixing 75 mL of 0.05 % w/v EDTA solution, 60 mL of 1 % w/v MPA solution and 15 mL of double distilled water (conductant = 17.4 mΩ) in a volumetric flask. Similarly, solvent B was prepared by mixing 75 mL of 0.05 % w/v EDTA solution, 60 mL of 1 % w/v MPA solution and 15 mL of 2 mM TCEP solution in a volumetric flask. Solvents A and B should be protected from direct light and stored at 4 °C when not in use. Next, two sets of 20-fold dilutions of ASE extracts were prepared using solvent A and B respectively. Briefly, for first set of dilutions using Solvent A, 50 µL aliquot of extract was mixed with 50 µL hipuric acid (internal standard, Molarity = 1 mg/mL) and 900 µL of solvent A in a centrifuge tube. The resulting mixture was incubated for 20 min in darkness at 0 °C and centrifuged afterwards (10,000 rpm, 5 min, 0°C). The supernatant was carefully collected into amber colored vials for HPLC-HRAMS analysis. A similar procedure was used to prepare a second set extract dilutions using solvent B. The extracted analytes were resolved on a Polar Acclaim II C18 column (150 × 4.6 mm I.D., particle size: 5 µm, pore diameter: 120 Å; Thermo Fisher Scientific, ON, Canada) coupled to a Dionex Ultimate 3000 ultra-high performance liquid chromatography (UHPLC) system and a LTQ Orbitrap high resolution accurate mass spectrometer (Thermo Fisher Scientific, ON, Canada). Formic acid solution (0.1 % v/v) was used as mobile under isocratic conditions for 13 min. The column temperature was set at 25 °C with a flow rate of 0.3 mL/min, and 5 µL of the sample or standards was injected in the instrument. The mass spectrometer was operated in ES1 negative ion mode using the following parameters: sheath gas: 40, auxiliary gas: 0, ion spray voltage: 3.0 kV, capillary temperature: 300 °C, capillary voltage: 100 V, Tube lens: -250 V, mass range: 60–500 m/z; full scan mode at a resolution of 60,000 m/z; top-3 data dependent MS/MS at a resolution of 3,000 m/z and step collision energy of 35 (arbitrary unit); acquisition time 13 min; isolation window: 2 m/z. The mass spectrometer was externally calibrated to 1 ppm using ESI negative calibration solutions (Thermo Scientific, MO, USA). All samples were run with four replications for analysis. The concentration of vitamin C in sample was determined based on standard curve developed from 1 mg/mL ascorbic acid stock solution, and the results expressed in µg ascorbic acid equivalent/g fresh weight (FW).

3.3.8. Vitamin B

Vitamin B contents in lettuce plant was determined based on vitamin (B_2, B_5, B_9) according to the Akhavan and Barzegar (2017) with little modification. Briefly, ASE extract was used for vitamin B analysis. Samples were prepared by taking the 100 µL of sample in a 2 mL centrifuge tube, 50 µL of hippuric acid (1 mg/mL) was added as internal standard with 750 µL of distilled water. Samples were mixed and was incubated in dark (4 ℃, 15 min). After incubation samples were vertex and centrifuged (3500 rpm, 2 ℃. 10 min). Supernatant was transferred to 2 mL LC-MS vial. The extracted analytes were resolved on a Polar Acclaim II C18 column (150 × 4.6 mm I.D., particle size: 5 µm, pore diameter: 120 Å; Thermo Fisher Scientific, ON, Canada) coupled to a Dionex Ultimate 3000 ultra-high performance liquid chromatography (UHPLC) system and a LTQ Orbitrap high resolution accurate mass spectrometer (ThermoFisher Scientific, ON, Canada). Formic acid solution (10 mM, 0.1 % v/v) was used as mobile under ramp conditions for 23 minutes. The solvents were used with following scheme: 0-20 min, 55% (v/v) B; 20-20.5 min, 55% (v/v) B; 20.5-23 min, 55%-0% (v/v) B. The column temperature was set at 35 °C with a flow rate of 0.3 mL/min, and 5 µL of the sample or standards was injected in the instrument. The mass spectrometer was operated in ES1 positive ion mode using the following parameters: sheath gas: 40, auxiliary gas: 0, ion spray voltage: 3.0 kV, capillary temperature: 300 °C, capillary voltage: 100 V, Tube lens: -250 V, mass range: 50–500 m/z; full scan mode at a resolution of 60,000 m/z; top-3 data dependent MS/MS at a resolution of 3,000 m/z and normalized collision energy of 35 (arbitrary unit); acquisition time 23 min; isolation width: 1 m/z. The mass spectrometer was externally calibrated to 1 ppm using ESI positive calibration solutions (Thermo Scientific, MO, USA). All samples were run with four replications for analysis. The concentration of vitamin B in sample was determined based on standard curve developed from 1 mg/mL stock solution of (B_2, B_5, B_9), and the results expressed in µg/g fresh weight (FW).

3.3.9. Polyphenol analysis

Polyphenols (chlorogenic acid, chicoric acid, luteolin, quercetin glucoside, quercetin glucuronide, quercetin malonyl) were analysed in lettuce based on the Gavrilova et al. (2011) with little modification. Briefly, ASE extract was used to analyse the polyphenols. 900 µL of ASE extract was measured in the 2 mL vial and placed in -80 °C freezer for 3h. Then, samples were taken out and placed in the freeze dryer (LABCONCO, FreeZone 2.5 Plus; Vacuum (0.051 torr), at - 90 °C). To a known amount of freeze-dried extracts (1-5 mg) in 2 mL vial, 40 µL of internal standard hippuric acid and 960 µL of 10 % (v/v) formic acid were added. The resultant mixture was transferred to centrifuge vial and vortexed until homogenized, incubated in the darkness for 30 min at 4 °C and then centrifuged (3000 rpm, 10 min, 0 °C). The supernatant was carefully transferred into amber colored vials for LC-MS analysis. The UHPLC-HRMS/MS analysis was conducted on LTQ Orbitrap XL mass spectrometer (Thermo Scientific, MO, USA) with an automated Dionex UltiMate 3000 UHPLC system controlled by Chromeleon software. A Luna C18 column (100 × 2.0 mm I.D., particle size: 3 µm, pore diameter: 100 Å) purchased from Phenomenex (CA, USA) was used for polyphenol separation. The solvent system used on the analytical column was as follows: Solvent A consisted of H_2O (containing 0.1 % v/v formic acid) and Solvent B consisted of acetonitrile (0.1 % formic acid) solution. Chromatographic separation was carried out at 25 °C (column oven temperature) with a flow rate of 0.3 mL/min, and 5 µL of the extract was injected in the machine. The following solvent gradient was used for separating the polyphenols: 0-2 min, 10-20% (v/v) B; 2-15 min, 20% (v/v) B; 15-20 min, 80-100% (v/v) B; 20-25 min, 100-0% (v/v) B; 25-35 min, 0% (v/v) B. The Orbitrap mass spectrometer was operated in the ESI negative mode. The following optimized parameters were used for the Orbitrap mass spectrometer, sheath gas flow rate : 25, auxiliary gas flow rate : 2, ion spray voltage: 5.0 kV, capillary temperature: 320 ^{0}C; S-lens RF:80 V; capillary voltage: 10 V, mass range:50-1500 m/z; full scan mode at a resolution of 60,000 m/z; top-6 data dependent MS/MS at a resolution of 30,000 m/z and collision energy of 35 (arbitrary unit); injection time 15 min; isolation window: 2.0 m/z; automatic gain control target: 2 e5 with dynamic exclusion setting of 30.0s. All samples were run with four replications for analysis. The concentration of vitamin polyphenols in sample was determined based on standard curve developed from 1 mg/mL stock solution of (chlorogenic acid, chicoric acid, luteolin, quercetin glucoside, quercetin glucuronide, quercetin malonyl), and the results expressed in µg/g fresh weight (FW).

3.3.10. Phenolic and antioxidant analyses

Sample extraction was carried out according to the Cano et al. (2003) with little modification. Briefly, 1 g lettuce leaf sample was weighed into centrifuge tube and mixed with 5 mL of 50 mM sodium phosphate buffer (pH 7.5) and the mixture homogenized with handheld homogenizer. Then mixture was incubated in darkness for 30 min and centrifuged (5500 rpm at 10 °C for 10 min). The supernatant pooled carefully without disturbing the pellet and collected as the hydrophilic extracts. The residue was re-suspended in 5 mL of 0.7 % acidified ethanol and vortexed to homogenize. The resultant mixture was incubated for 30 min in darkness then centrifuged (5500 rpm at 10 °C for 10 min). The supernatant was carefully collected as lipophilic extracts. The lipophilic and hydrophilic extracts were stored in darkness at – 20 °C.

3.3.11. Total antioxidant analysis

Ferric reducing ability of plasma (FRAP) method was used to determine the hydrophilic antioxidant content (HAA) and lipophilic antioxidant content (LAA) of lettuce leaf samples as described by Benzie and Strain (1996) with slight modifications. Briefly, FRAP working solution was prepared freshly by mixing 25 mL of sodium acetate buffer solution (pH= 3.6; molarity = 300 mM), 2.5 mL of TPTZ solution (2,4,6-tripyridyl-s-triazine ; Molarity = 10 mM) and and 2.5 mL of FeCl3.6H20 solution (ferric (III) chloride hexahydrate ; Molarity = 20 mM). The resulting mixture was incubated at 37 ℃ for 15 min before use. Then 20 µL of sample extract was mixed with 180 µL of FRAP working solution in a 96-well microplate. The mixture was incubated for 30 minutes in darkness and the absorbance of the resulting colored mixture read at 593 nM using the cytation imaging microplate reader. The concentration of antioxidants in sample extracts was determined based on standard curve developed from 1 mM Trolox stock solution, and results expressed in µM Trolox equivalents (TE)/g fresh weight (FW). Values for total antioxidant content (TAA) were determined by adding HAA and LAA values.

3.3.12. Total Phenolics

Total phenolic content in lettuce leaves was determined using the Folin–Ciocalteu method as described by Cano et al. (2003) with slight modifications. Briefly, 10-fold diluted working solution was prepared by mixing 5 mL of Folin–Ciocalteu reagent and 45 mL of distilled water. For samples, 25 µL of appropriate sample extract were mixed with 125 µL of 10-fold Folin–Ciocalteu working solution and 50 µL of sodium phosphate buffer (pH = 7.5 ; Molarity = 50 mM) or 0.7 % acidified ethanol respectively depending on whether hydrophilic or lipophilic sample extracts are being analyzed. The microplates were incubated for 30 min in darkness and absorbance measured at 755 nm on a Cytation imaging microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). The concentration of total phenolic in sample extracts was determined based on standard curve developed from 1 mg/mL Quercetin stock solution, and the results expressed in mg Quercetin equivalent (QE)/g fresh weight (FW). Values for total phenolic contents (TPC) were determined by adding HPC and LPC values.

3.4. Statistical analysis

Two-way analysis of variance (ANOVA) was employed to determine the effects of experimental treatments on minerals, vitamins, phenolics and antioxidants in lettuce leaves using the Statistix-10 (Analytical Software, FL, USA). Where treatment effects were significant, the means were compared with Fisher's Least Significant Difference (LSD) at alpha 0.05. Prior to data analysis normality was tested using Shapiro-Wilkes test. Figures were prepared using SigmaPlot 13.0 software program (Systat Software Inc., San Jose, CA, USA) and heatmaps were prepeared using the XLState (60987; Addinsoft, FR, Paris).

3.5. Results

3.5.1. Minerals concentrations

Mineral feed solution (MFS), varieties (V) and their interaction had significant ($p<0.05$) effects on minerals in lettuce. MFS and V had significant effects on P, K, Ca, Mg, Fe, and Cu, whereas, interactive effects of MFS × V had significantly influenced N, S, B, Zn and Mn concentration in lettuce leaves (Figure 9 A-F) and 10 (A-E)). Individual comparison of treatment means showed that NS exhibited higher P concentration (6.41 mg g^{-1}) compared to lower P (2.85 mg g^{-1}) was recorded in DD (Figure 9B). However, Romaine showed 45% higher P uptake than Newham.

Likewise, higher K, Ca, Mg, Fe and Cu was observed in lettuce when grown in NS and lowest were observed when cultivated in DD solution. Uptake of K, Ca, Mg, Fe and Cu was significantly higher in Romaine than in Newham.

NS and Romaine showed higher N uptake (47.50 mg g^{-1}), followed by NS + DD and Romaine (42.31 mg g^{-1}), and lowest N uptake (38.11 mg g^{-1}) was observed in DD and Newham. Romaine showed superior performance and exhibited higher N uptake irrespective of nutrient solution, whereas, lower N uptake (Figure 9 A) was observed in Newham. Higher S (2.86 mg g^{-1}) was noted in NS and Romaine, followed by NS + DD and Romaine (2.24 mg g^{-1}), and lowest (1.53 mg g^{-1}) was observed in DD and Newham (Figure 9 E). Boron uptake was higher in NS and Romaine followed by NS and Newham. However, B uptake was statistically at par in both varieties when cultivated in NS + DD, and DD solution (Figure 10 C). Similarly, Zn uptake was higher in NS and Romaine, followed by NS and Newham and the lowest was recorded in Newham and DD treatment (Figure 10 D).

Interestingly, Zn uptake in Romaine and Newham grown in NS + DD and Romaine cultivated in DD were statistically non-significant (Figure 10 D). Figure 3E showed significantly higher Mn uptake in NS and Romaine, followed by Newham and NS treatment whereas, significantly lower Mn uptake was noted in DD and Newham treatment, though statistically at par with DD and Romaine and NS + DD and Newham (Figure 10 E). Again, Romaine performed better than Newham and showed higher Mn uptake irrespective of nutrient solution (Figure 10 E).

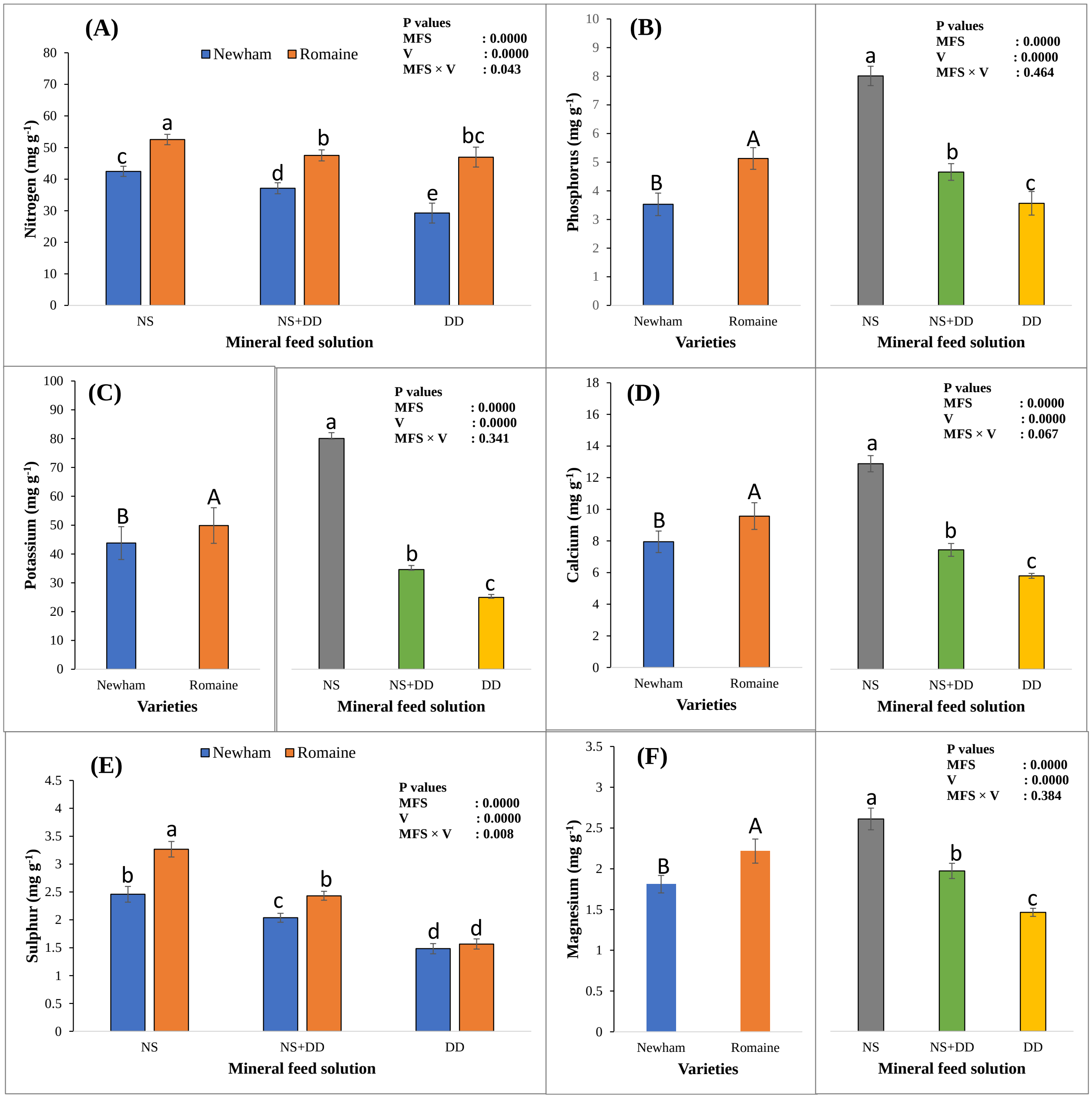

Figure 9: Effects of DD and inorganic nutrient solution on minerals uptake; nitrogen (A), phosphorus (B), potassium (C), calcium (D), sulfur (E) and magnesium (F) concentration in lettuce cultivated in hydroponic system. Vertical bars are means of three replications ±SE. Bars sharing the same letter, for individual factors and interaction, do not differ significantly at LSD ≤ 0.05; NS = nutrient solution; NS+DD = 50% NS + 50% DD; DD = dairy digestate; MFS= mineral feed solution; V = varieties; MFS × V = interaction between mineral feed solution and varieties

3.5.2. Crude protein (%)

Statistical analysis showed that MFS, V and interactive effects of MFS × V had significant effects on crude protein in lettuce leaves. Data presented in Figure 10 F showed 33 % crude protein in NS and Romaine which was higher than Romaine and NS+DD (29.70 %) and significantly lower (18.28 %) in DD × Newham. Romaine × NS+DD produced statistically similar crude protein of Newham × NS treatment. On the other hand, crude protein was 12 % and 25 % higher in NS than NS+DD and DD treatment. Whereas, Romaine exhibited 35 % higher crude protein than Newham (Figure 10 F).

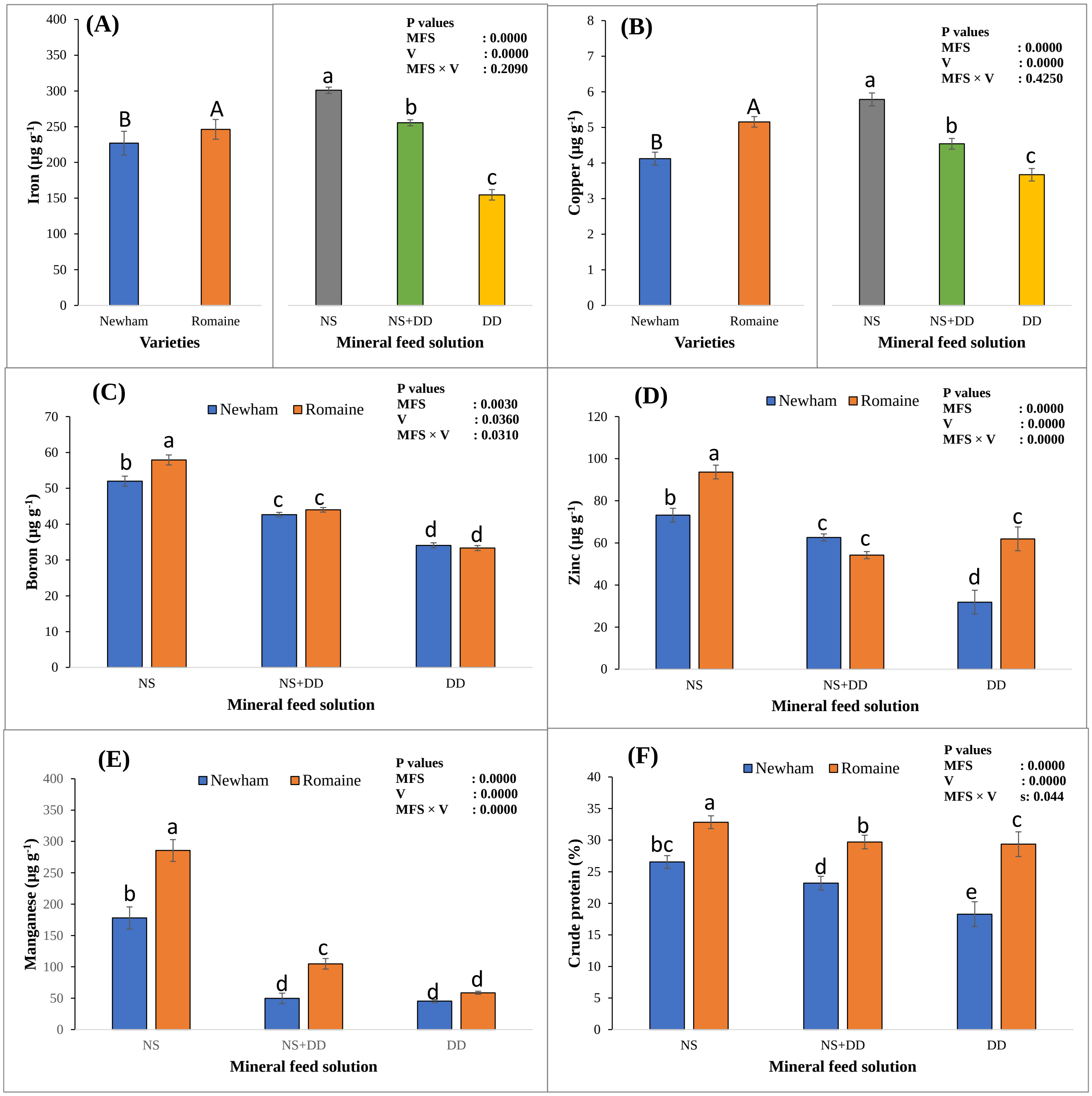

Figure 10: Effects of DD and inorganic nutrient solution on uptake of iron (A), copper (B), boron (C), zinc (D), manganese (E) and crude protein contents (F) in lettuce grown in hydroponics. Vertical bars are means of three replications ±SE. Bars sharing the same letter for individual factors and interaction, do not differ significantly at LSD ≤ 0.05; NS = nutrient solution; NS+DD = 50% NS + 50% DD; DD = dairy digestate; MFS = mineral feed solution; V = varieties; MFS × V = interaction between mineral feed solution and varieties

3.5.3. Vitamins

Results indicated that MFS, V and their interaction had significant ($p<0.05$) effects on lettuce vitamins. However, MFS and V had significant effects on pantothenic acid, whereas, interactive effect of MFS × V had significantly ($p<0.05$) influenced vitamin C, riboflavin and folates concentration in lettuce leaves (Figure 11 A-D). NS and Romaine produced higher vitamin C (8.09 µg g^{-1} FW), followed by NS × Newham and lowest (0.68 µg g^{-1} FW) was observed in DD and Romaine. Vitamins C in both Newham and Romaine varieties were statistically non-significant when cultivated in NS+DD and DD treatments (Figure 11 A). Similarly, riboflavin concentration was higher (0.006 mg g^{-1}) in NS and Romaine followed by the NS and Newham (0.004 mg g^{-1}) and lowest (0.002 mg g^{-1}) was observed in DD and Newham (Figure 11 B). Riboflavin production by Romaine and Newham were statistically non-significant when grown in DD solution.

On the other hand, DD solution produced higher (0.03 mg g^{-1}) pantothenic acid and lower (0.02 mg g^{-1}) was found in NS, but statistically at par with NS+DD treatment (Figure 11 C). Similarly, DD and Romaine exhibited significantly higher folates contents (757 µg g^{-1}) and lowest (178 µg g^{-1}) was observed in NS and Newham, though statistically at par with all treatments except DD × Romaine (Figure 11 D).

3.5.4. Total phenolics and total antioxidants

Data presented in Figure 11 E indicated that MFS, and V had significant ($p<0.05$) effects on total antioxidants. Individual comparison of treatment means exhibited that DD treatment produced higher (33.44 mg g-1 sample FW) total antioxidants in lettuce, followed by NS + DD treatment whereas, NS treatment produced significantly lower (23.21 mg g-1 sample FW) total antioxidants.

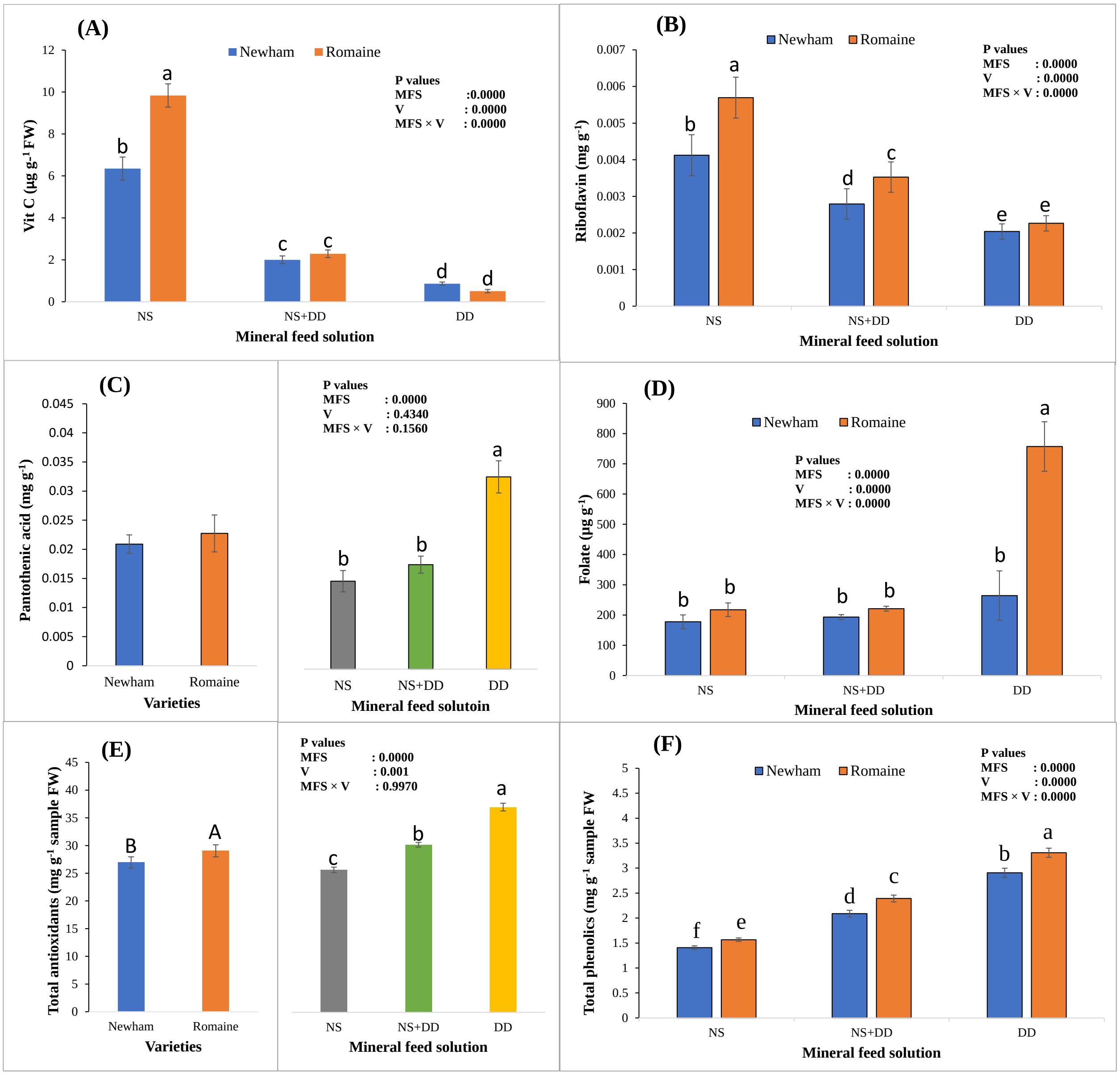

Figure 11: Effects of DD and inorganic nutrient solution on vitamin C (A), riboflavin (B), pantothenic acid (C), folate (D), total antioxidants (E) and total phenolics (F) concentration of lettuce in hydroponic system. Vertical bars are means of three replications ±SE. Bars sharing the same letter, for individual factors and interactive effects, do not differ significantly at LSD ≤ 0.05; NS = nutrient solution; NS+DD = 50% NS + 50% DD; DD = dairy digestate; MFS= mineral feed solution; V= varieties; MFS × V= interaction between mineral feed solution and varieties

Romaine produced approximately 8 % higher total antioxidants than Newham (Figure 11 E). Total phenolics in lettuce leaves were significantly influenced by the interactive effects of MFS × V

(Figure 11 F). Data presented in Figure 11 F showed higher total phenolics (3.31 mg g^{-1} sample FW) in DD × Romaine, followed by DD × Newham (2.91 mg g^{-1} sample FW), whereas, NS × Newham produced lower total phenolic contents (1.41 mg g^{-1} sample FW). Total phenolic production from NS + DD treatment was lower than DD and higher than NS treatment (Figure 11 F).

3.5.5. Phenolic acid and flavonoids

MFS had significant ($p<0.05$) effects on chlorogenic acid, whereas, MFS and V significantly influenced chicoric acid, and quercetin-3-β-d-gluconide (Figure 12A, B&F). Interaction of MFS × V showed significant effects on luteolin, quercetin-3-glucoside, and quercetin-β-malonyl concentration in lettuce leaves (Figure 12 C- E). Data presented in Figure 12 A revealed that lettuce grown in DD showed higher (0.005 mg g^{-1} DW) chlorogenic acid followed by NS + DD and lowest (0.002 mg g^{-1} DW) was observed in NS treatment. Likewise, DD treatment produced higher (0.053 mg g^{-1} DW) chicoric acid compared to NS treatment which produced lowest (0.029 mg g^{-1} DW), though statistically at par with NS + DD treatment. Romaine produced 94% higher chicoric acid than Newham (Figure 12 B).

DD × Romaine interaction exhibited higher luteolin concentration (0.0009 mg g^{-1} DW) followed by the DD and Newham (0.0007 mg g^{-1} DW) and lowest luteolin concentration (0.0001 mg g^{-1} DW) was observed in NS × Newham treatment combination. Interactive effects of NS + DD × Romaine and Newham × NS + DD treatments produced statistically similar luteolin (Figure 12 C). Higher quercetin-3-glucoside (0.003 mg g^{-1} DW) was noted in DD and Romaine, followed by DD and Newham (0.002 mg g^{-1} DW), and lowest (0.0003 mg g^{-1} DW) was observed in NS and Newham (Figure 12 D). Quercetin-3-glucoside production was statistically similar in both varieties when grown in NS+DD treatment (Figure 12 D). DD and Romaine produced significantly higher quercetin-β-malonyl (0.212 mg g^{-1} DW) followed by NS + DD and Romaine whereas, NS × Newham treatment produced lowest (0.004 mg g^{-1} DW) (Figure 12 E). It is pertinent to mention here that the gap in higher and minimum quercetin-β-malonyl production among treatments was significantly wider. Romaine produced higher quercetin-β-malonyl than Newham when cultivated in DD and NS + DD treatments, but, statistically at par with Newham when grown in NS treatment (Figure 12 E).

Individual comparison of treatment means showed that lettuce grown in DD solution displayed higher (0.086 mg g^{-1} DW) concentration of quercetin-3-β-d-gluconide followed by NS + DD and lowest (0.018 mg g^{-1} DW) was observed in NS treatment. Romaine produced 64% higher quercetin-3-β-d-gluconide than Newham (Figure 12 F).

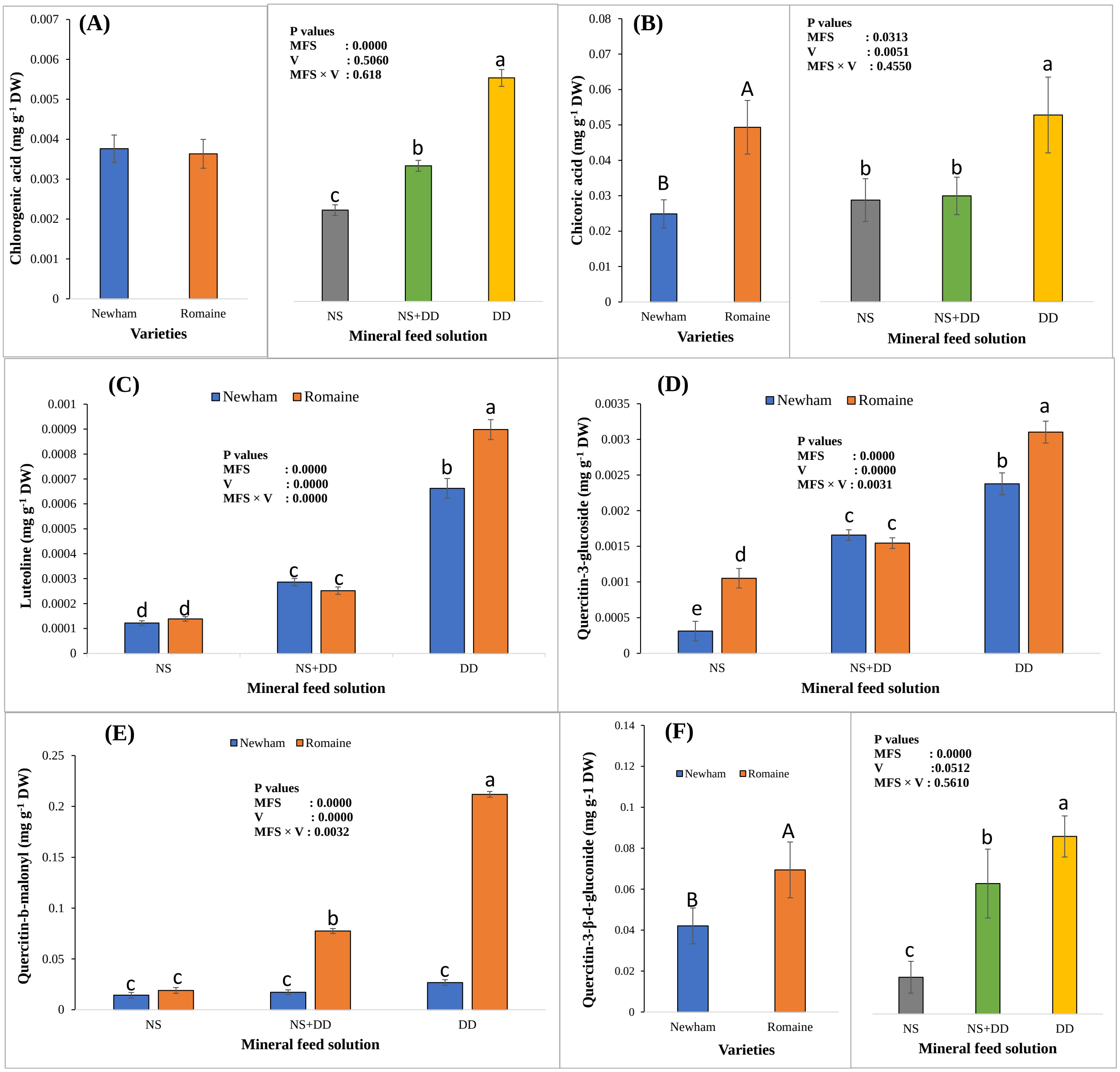

Figure 12: Effects of DD and inorganic nutrient solution on chlorogenic acid (A), chicoric acid (B), luteolin (C), quercetin-3-glucoside (D), quercetin-β-malonyl (E) and quercetin-3-β-d-gluconide (F) in lettuce cultivated in hydroponics. Vertical bars are means of three replications ±SE. Bars sharing the same letter, for individual factors and interaction, do not differ significantly at LSD ≤ 0.05; NS = nutrient solution; NS+DD = 50% NS + 50% DD; DD = dairy digestate; MFS= mineral feed solution; V= varieties; MFS × V = interaction between mineral feed solution and varieties

3.6. Discussion

Leafy vegetables are chief source of minerals required for human health; however, mineral concentration in leafy vegetables may change due to nutrients availability from substrate (Silber and Bar-Tal 2008). Hassan et al. (2012) observed that organically grown *Cosmos caudatus* contained lower concentration of minerals than when grown on inorganic nutrient solution, as probably nutrients are bound with organic material result in slow release (Benbrook et al. 2008). Another study conducted by Kapoulas et al. (2017), also reported lower minerals concentration in organically grown lettuce and onion than inorganic source. In present study, we also observed lower minerals in lettuce cultivated in DD solution compared to higher minerals in NS (Figure 9; 10 & 15). Higher NH_4^+- N and pH in DD may cause ionic imbalance and reduce uptake of essential cations such as K^+, Ca^{2+} and Mg^{2+} (Barker et al. 1967; Gerendás et al. 1997; Roosta and Schjoerring 2007). In present study, we also found lower concentration of K^+, Ca^{2+} and Mg^{2+} in the lettuce leaves when grown in DD (Figure 9C; 9D; 9F & 15). Zandvakili et al. (2019) investigated the effects of organic and inorganic solution on four lettuce cultivars. They suggested that lettuce grown in inorganic solution tends to have higher concentration of N, K, and Ca than organically fed lettuce. They further noticed variation in minerals among different cultivars, for example, Arroyo cultivar showed higher minerals than other lettuce cultivars. Mou and Ryder (2004) observed variation in lettuce minerals due to difference in head morphology. Lettuce with loose head morphology showed higher minerals compared to lower minerals in tighter head (Zandvakili et al. 2019). In present study, we observed Romaine variety with loose head morphology showed higher minerals, whereas, Newham with tighter head morphology produced lower minerals (Figure 9; 10; 13 & 15). Higher and lower minerals phenomenon in loose and tight head morphology of lettuce in organic and inorganic nutrient supply in hydroponics is not clear which needs further investigation.

Figure 13: Growth pattern/head morphology of Newham (a) and Romaine (b) cultivated in different nutrient solutions.

Contrarily, it was reported that organically grown cabbage and lettuce has higher Mn and Zn concentration compared to conventionally grown lettuce (Warman and Havard 1997; Kapoulas et al. 2017). Whereas, Zandvakili et al. (2019) observed that lettuce grown in organic solution has lower concentration of Zn and higher Mn uptake compared to inorganically grown lettuce. While, in present study we observed lower concentration of Mn, Zn and B in lettuce leaves when grown in DD (Figure 10 D & 10 E). Savvas et al. (2006) argued that higher NH_4^+-N tends to increase the digestate pH, mainly regulated by $NH_4^+ \leftrightarrow NH_3$, $CO_2 \leftrightarrow HCO_3^- \leftrightarrow CO_3^{2-}$, and $CH_3COOH \leftrightarrow CH_3COO^-$ species (Sommer and Husted 1995b; Hjorth et al. 2010). Generally, pH is increased due to formation of ammonium carbonate $((NH_4)_2CO_3$ (Georgacakis et al. 1982; Webb and Hawkes 1985) and removal of CO_2 (Sommer and Husted 1995a). This increased in pH might reduced the availability of Mn, Cu and Zn in the lettuce leaves (Savvas et al. 2006). Increased solution pH from 6.0 to 7.0 decreased solubility and uptake of Mn and Zn in plants (Adams 2002). Lower concentration of these micronutrients in lettuce grown in DD in present study could be attributed to lower mineral nutrient composition (Table 4) and higher pH of DD (Figure 14), might have reduced solubility of nutrients resulted in low uptake of these micronutrients. Research conducted by Nicoletto et al. (2014) reported the lower N, P (16.7 – 21.9 mg g^{-1}) and higher K (37.8 – 48.0 mg g^{-1}) in lettuce grown in anaerobic digestate than inorganic nutrient source. In an other study conducted by Przygocka-Cyna et al. (2018) evaluated minerals in biogas digestate and combination of inorganic and biogas digestate and observed higher concentration of minerals in biogas digestate. Minerals observed were, for example, Ca ranges from 9.8 – 13.4 mg g^{-1}, Mg (1.9 – 2.7); Mn (143 – 354 µg g^{-1}), Fe (209 – 386 µg g^{-1}), Zn (137 – 225) and Cu (6.3 – 8.7 µg g^{-1}), respectively. These studies endorsed the findings of present study where we observed similar mineral concentration and were well within the range which suggest that though nutrients were low in DD compared to NS but not deficient to a level to restrict the growth and yield of lettuce. Seefeldt (2015) observed nutrients sufficiency range in lettuce and reported N; 35 – 50 mg g^{-1}, P; 4.0 – 8.0 mg g^{-1}, K; 55 – 96 mg g^{-1}, Ca;14 – 28 mg g^{-1}, Mg; 3.6 – 8.0 mg g^{-}, B; 25 – 60 µg g^{-1}, Cu; 5 – 25 µg g^{-1}, Fe; 40 – 100 µg g^{-1}, Mn; 11 – 250 µg g^{-1} and Zn was 20 – 250 µg g^{-1}. Nutrients concentaration in lettuce tissues cultivated in DD solution in present study were; N (22.1 – 50.5 mg g^{-1}, P (1.2 – 4.3 mg g^{-1}), K (22.3 – 30.4 mg g^{-1}), Ca (5.2 – 6.8 mg g^{-1}), Mg (1.1 – 1.9 mg g^{-1}); B (31 – 38 µg g^{-1}), Cu (3 – 4.5 µg g^{-1}), Fe (118 – 186 µg g^{-1}), Mn (40 – 69 µg g^{-1}) and Zn (30 – 84 µg g^{-1}) which were within sufficient range and suggest that DD could be used as organic fertilizer source.

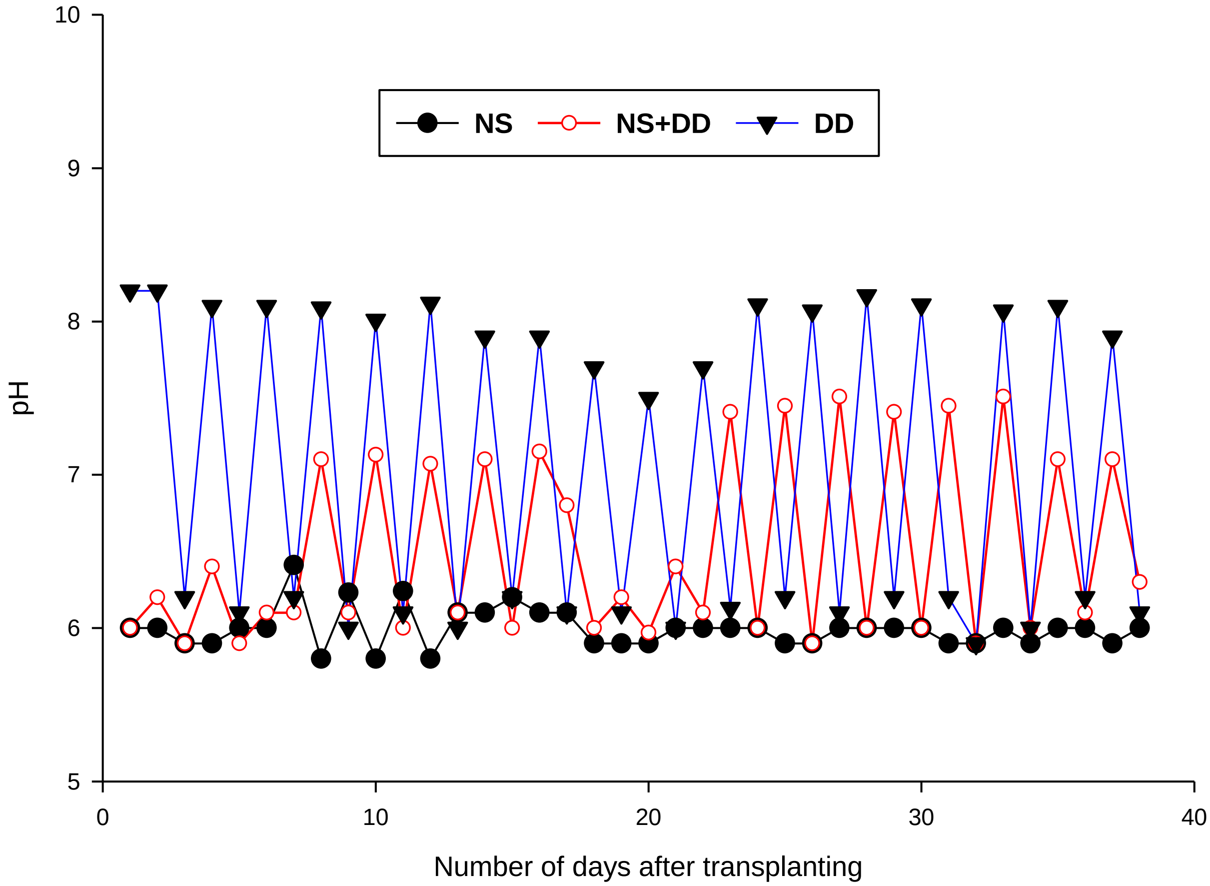

Figure 14: Variation in nutrient solutions pH over entire growing season of lettuce. NS = nutrient solution; NS+DD = combination of nutrient solution and dairy digestate; DD = dairy digestate

Crude protein concentration is directly related to the total N contents of the leaves. Higher N concentration in solution can enhance N uptake in plants and ultimately crude protein (Bryson et al. 2014). In the present study, NS treatment increased N uptake by 12% and 25% compared to NS+DD and DD treatments (Figure 9 A) and consequently higher crude protein (Figure 10 F). Our results are in agreement with that reported by (Zandvakili et al. 2019), who observed high crude protein in inorganically grown lettuce compared to organically fed plants.

Vitamins are the naturally occurring organic compounds in the leafy vegetables and are required for proper metabolism (Survase et al. 2006). Additionally, vitamins are good food additives, and are considered important medical therapeutic agents (Kim et al. 2016). Previous researchers reported that organic nutrient source such as biogas slurry increased vitamin C content in bokchoi (Liu et al. 2006), cucumber (Hao et al. 2007) and in lettuce (Su et al. 2008). Whereas, Xu et al. (2003a, b) observed lower vitamin C concentration in lettuce either grown in organic fertilizer or in agro-industrial waste compost and higher vitamin C with inorganic N fertilized lettuce. Lower vitamin C observed in lettuce grown in DD in present study could be due to higher NH_4^+-N in solution (Table 4) which might have diminished vitamin C production (Mapson and Cruickshank 1947) or higher concentration of NH_4^+ triggers the reversible production of GDP-mannose which is key intermediate enzyme required for vitamin C synthesis (Bonin et al. 1997; Conklin et al. 1999b). Our results corroborate the findings of previous studies reporting lower vitamins C in lettuce and Kacip Fatimah (*Labisia pumila* Benth) when cultivated in organic nutrient source (Ibrahim et al. 2013). We observed higher vitamin C (8.09 µg g^{-1} FW) in NS treatment and lower in DD treatment (Figure 11 A).

Riboflavin, pantothenic acid and folates are also important vitamins for the normal functioning of human body. Watson and Noggle. (1947) investigated the effect of different mineral feed solutions (deficient in one or more nutrients) on riboflavin contents in oat plant (*Avena sativa* L). mineral feed solutions were Hoagland solution as a control and other treatments were deficient in one particular mineral such as K, Ca, Mg, NO_3^-, SO_4^{2-} and PO_4^{3-}.They observed that mineral deficiencies affected riboflavin synthesis and found a positive relationship between N uptake and riboflavin synthesis in oats leaves. In the present study, we observed lower riboflavin contents, higher pantothenic acid and folates in DD grown lettuce (Figure 11 (B-D)). Lower riboflavin contents in the present study might be due to NO_3^--N deficiency or lower NO_3^-/NH_4^+ ratio in DD treatment which reduced riboflavin contents in lettuce. Additionally, N-uptake in lettuce grown in

DD was significantly lower than NS treatment (Figure 9 A), which support the arguments of Watson and Noggle (1947).

Likewise, lettuce varieties exhibited variation in folate contents and other vitamins (Kim et al. 2016) and was also consistent with studies conducted by Mou (2009) who reported Romaine leaf contains higher concentration of riboflavin, pantothenic acid and folates contents than crisphead variety. He reported that these vitamins concentration ranges from 0.03 mg - 0.08 mg/100g sample for riboflavin, 0.09 - 0.15 mg/100g sample for pantothenic acid and 29 - 136 µg/100g sample for folates. In present study, riboflavin, and pantothenic acid were lower (0.002-0.005 mg g^{-1} sample, 0.015-0.033 mg g^{-1} sample) and folate contents were much higher (197.62-510.91 µg g^{-1} sample) as observed by Mou (2009) (Figure 11B, 11C & 11D).

Total phenolics and total antioxidants were significantly influenced by mineral nutrient sources. DD application (organic source) enhanced total phenolics and total antioxidants compared to NS (inorganic source) treatment (Figure 11E & 11F). Ibrahim et al. (2013) reported higher total phenolics and total antioxidants in Kacip Fatimah (*Labisia pumila* Benth) grown in chicken dung (10: 10: 10 NPK) compared to inorganic fertilizers (15: 15: 15 NPK). Higher total phenolics and antioxidants in organic fertilizer could be due to slow or limited N availability, which changes metabolism towards carbon-containing compounds and non-N-containing secondary metabolites such as phenolics and terpenoids (Haukioja et al. 1998). In present study, we observed higher phenolics and total antioxidants in lettuce grown in DD which can be attributed to lower N availability compared to NS (Table 4). Results of our study are consistent with studies reported by Ibrahim et al. (2013) and Haukioja et al. (1998). Liu et al. (2007) investigated twelve green and red lettuce cultivars for their phenolic contents and reported higher total phenolic contents in red type than green type of lettuce which varied from 21-74 mg g^{-1} sample FW. Similarly, Mampholo et al. (2016) also found significant variation in total antioxidants (15 -127 mg TEAC (Trolox equivalent antioxidant capacity) $100g^{-1}$ FW) production among the lettuce varieties. In present study, total phenolic and total antioxidants contents in lettuce varieties varied from 1.4 - 3 mg g^{-1} sample FW and 22 - 34 mg g^{-1} sample FW respectively (Figure 11E & 11F).

Phenolic acid such as chicoric acid has immune-stimulatory properties, promote phagocytosis activity (Bauer et al. 1989b) and also helpful to inhibit the HIV replication (Healy et al. 2009b), whereas, chlorogenic acid is helpful against hepatitis B virus in human (Zuo et al. 2015). Quercetin and their derivatives, found in fruits and vegetables, have unique biological properties that may not only improve mental and physical health but also reduce infection risk (Davis et al. 2009). Several studies reported that polyphenols have anti-inflammatory and therapeutic properties that may reduce cardiovascular disease, neurodegenerative disorders, cancer, and obesity (Pérez-Jiménez et al. 2010). Biosynthesis of polyphenolic compounds is highly dependent on N fertilization practices and a lower availability of N is associated with increased phenolic compounds. For example, in a recent study conducted by Mollavali et al. (2018) reported increased concentration of various polyphenols such as quercetin-4′-O-β-D-glucoside and of quercetin-3,4′-di-O-β-D-glucoside in onion plants. This increased polyphenol concentration is closely associated with increased phenylalanine ammonia lyase (PAL) enzyme activity in the plants grown in predominantly higher NH_4^+-N solution. Higher NH_4^+-N might have stimulated biosynthesis of phenolic compounds and increased expression of soluble peroxidase and superoxide dismutase enzyme under oxidative stress condition (Mihaljević et al. 2011). In present study, higher NH_4^+-N in DD solution might have triggered a stress response and stimulated PAL enzyme that enhanced biosynthesis of polyphenols and total antioxidants (Figure 11 (E-F), 12(A-F) and 15). Similarly, Durazzo et al. (2014) also observed higher concentration of polyphenols such as Lutein and Caffeic acid and lower chlorogenic acid and Quercetin in organically grown lettuce. Likewise, Rajashekar et al. (2012) also reported higher concentration of polyphenols concentration in organically grown lettuce as compared to conventionally grown lettuce. They also observed higher concentration of polyphenols such as chicoric acid (0.2 - 1.1 mg g^{-1} FW), chlorogenic acid (0.15 – 0.5 mg g^{-1} FW) and quercetin 3-O-glucoside (0.03-0.10 mg g^{-1} FW) in lettuce grown organically, whereas, Luteolin-7-O-glucoside (0.01 – 0.02 mg g^{-1} FW) was higher in conventionally grown lettuce. In present study, we also observed significant variation in Chicoric acid, Chlorogenic acid, Luteolin 3-O-glucoside and Quercetin 3-O-(malonyl)-b-D-glucoside concentration in the lettuce grown in DD than inorganically fertilized lettuce (Figure 12).

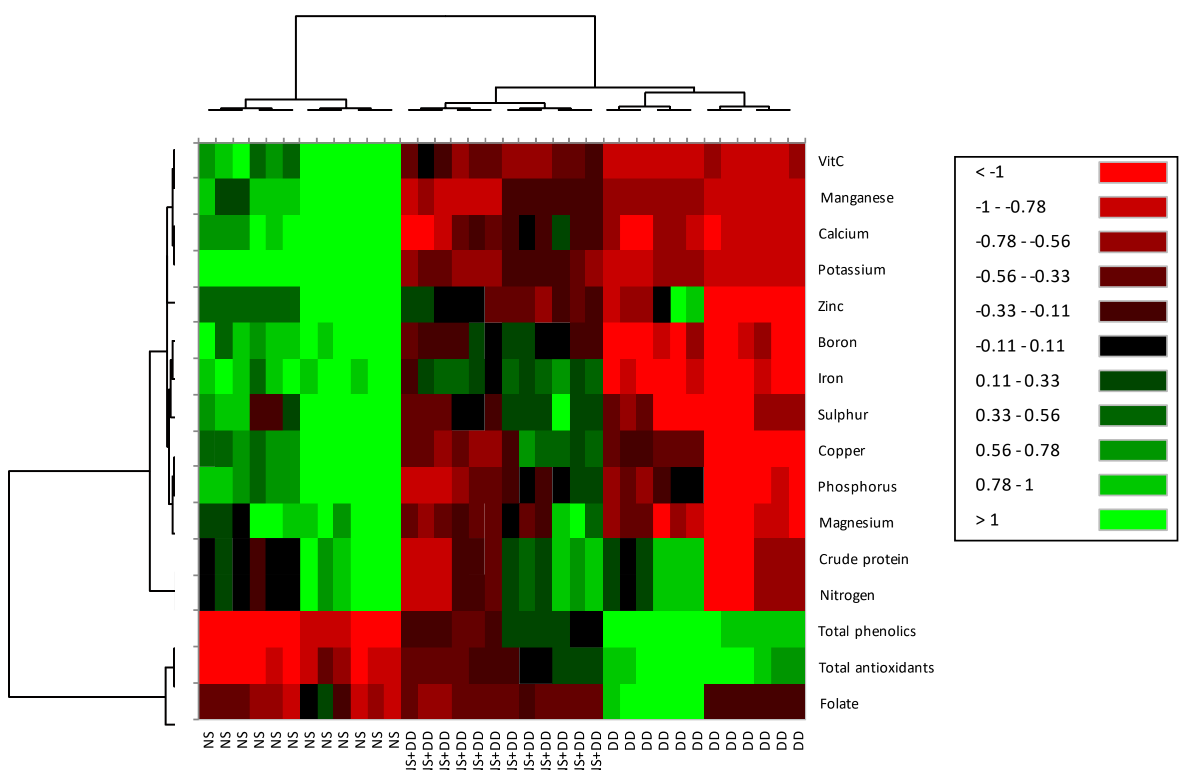

Figure 15: Heatmap displaying higher concentration of vitamin C, minerals, micronutrients and crude protein in NS treatment whereas, higher total phenolics, total antioxidants and folate in DD solution. Rows clearly segregated total phenolics, and antioxidants in one cluster, whereas, vitamin C, minerals and crude protein are clustered in another group. Likewise, mineral nutrients solutions are clustered in three distinct groups/columns (NS, NS + DD and DD). In the color key, red

represents the lowest whereas green represents the highest value for accumulated minerals, crude protein, total phenolics, total antioxidants and vitamins

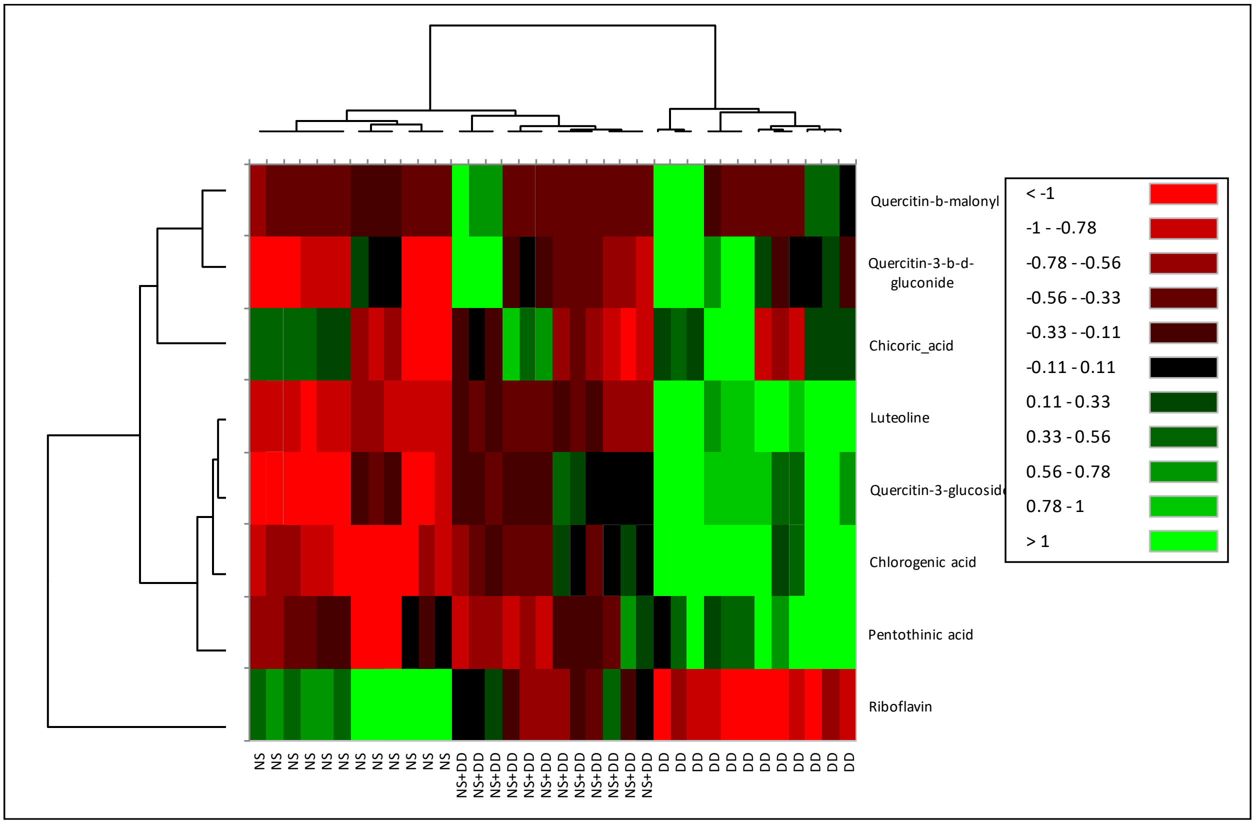

Figure 16: Heatmap displaying the clear segregation of polyphenols and riboflavin concentration in lettuce grown in different mineral feed solution. DD solution showed higher concentration of polyphenols whereas, NS displayed higher riboflavin. In the color key, red represents the lowest whereas green represents the highest value for accumulated polyphenols and riboflavin

3.7. Conclusion

Lettuce cultivation in DD nutrient solution in hydroponics could be one of the sustainable solutions to meet the challenges of food security amidst climate change and decreasing land resources. The present study highlighted dairy digestate as a sustainable nutrient source for improvement of phytochemical profile of lettuce in hydroponics. Results showed that NS (modified Hoagland solution) produced higher minerals and vitamins C in lettuce whereas, DD showed superior performance as a sole nutrient source and exhibited higher vitamins (riboflavin, pantothenic acid folates), phenolics, flavonoids (chicoric acid, Chlorogenic acid, Quercetin-3-β-d-gluconide, luteolin, quercetin-3-glucoside and quercetin-β-malonyl), and total antioxidants. This research demonstrates that DD could be used as sustainable nutrient source to improve phytochemical profile of lettuce in hydroponics production system.

3.8. References

Adams P (2002) Nutritional control in hydroponics. In: Savvas, D., Passam, H.C. (Eds.), Hydroponic Production of Vegetables and Ornamentals. Embryo Publications, Athens, Greece,

Ahmad R, Jabeen N (2009) Demonstration of growth improvement in sunflower (helianthus annuus l.) By the use of organic fertilizers under saline conditions

Ahring B (2003) Preface. In: Ahring BK (ed) Biomethanation II. Springer, Berlin.

Akhavan HR, Barzegar M (2017) Determination of water-soluble vitamins in 15 Iranian pomegranate cultivars and their variation after pasteurization and cold storage. . Int Food Res J 24:1429

Alburquerque JA, de la Fuente C, Ferrer-Costa A, et al (2012) Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass and bioenergy,. Elsevier 40:181–189

Arnon DI (1949) Copper enzymes in isolated chloropolasts. polyphenoloxidase in (Beta vulgaris). Plant Physiol 1–15

Bachmann S, Uptmoor R, Eichler-Löbermann B (2016) Phosphorus distribution and availability in untreated and mechanically separated biogas digestates. Sci Agric 73:9–17

Bachmann S, Wentzel S, Eichler-Löbermann B (2011) Codigested dairy slurry as a phosphorus and nitrogen source for Zea mays L. and Amaranthus cruentus L. J Plant Nutr Soil Sci 174:908–915

Barker A V., Eaton TE, Meagy MJ, et al (2017) Variation of mineral nutrient contents of modern and heirloom cultivars of cabbage in different regimes of soil fertility. J Plant Nutr 40:2432–2439

Barker A V., Maynard DN, Lachman WH (1967) Induction of tomato stem and leaf lesions, and potassium deficiency, by excessive ammonium nutrition. Soil Sci 103:319–327

Barth C, Gouzd ZA, Steele HP, Imperio RM (2010) A mutation in GDP-mannose pyrophosphorylase causes conditional hypersensitivity to ammonium, resulting in Arabidopsis root growth inhibition, altered ammonium metabolism, and hormone homeost. J Exp Bot 61:379-394.

Barzee TJ, Edalati A, El-Mashad H, et al (2019) Digestate biofertilizers support similar or higher tomato yields and quality than mineral fertilizer in a subsurface drip fertigation system. Front Sustain Food Syst 3:58

Bauer A, Mayr H, Hopfner-Sixt K, Amon T (2009) Detailed monitoring of two biogas plants and mechanical solid–liquid separation of fermentation residues. J Biotechnol 142:56–63

Bauer R, Reminger P, Jurcic K, Wagner H (1989a) Influence of Echinacea extracts on phagocytic activity. Z Phytother 10:43–48

Bauer R, Reminger P, Jurcic K, Wagner H (1989b) Influence of Echinacea extracts on phagocytic activity. Z Phytother 10:43–48

Benbrook C, Zhao X, Yáñez J, et al (2008) New Evidence Confirms the Nutritional Superiority of Plant-Based Organic Foods. State of Science Review; The Organic Center: Boulder, CO, USA,

Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal Biochem 239:70–76

Bittsánszky A, Pilinszky K, Gyulai G, Komives T (2015) Overcoming ammonium toxicity. Plant Sci 231:184–190

Bloch D, Monshausen G, Singer M, et al (2011) Nitrogen source interacts with ROP signalling in

root hair tip-growth. Plant, Cell Environ 34:76–88

Bonin CP, Potter I, Vanzin GF, Reiter W-D (1997) The MUR1 gene of Arabidopsis thaliana encodes an isoform of GDP-D-mannose-4, 6-dehydratase, catalyzing the first step in the de novo synthesis of GDP-L-fucose. Proceedings of the National Ac. Plant Biol 94:2085–2090

Boonpangrak S, Lalitmanat S, Suwanwong Y, et al (2016) Analysis of Ascorbic Acid and Isoascorbic Acid in Orange and Guava Fruit Juices Distributed in Thailand by LC-IT-MS/MS. Food Anal Methods 9:1616–1626

Börjesson P, Berglund M (2007) Environmental systems analysis of biogas systems—Part II: The environmental impact of replacing various reference systems. Biomass and Bioenergy 31:326–344

Bottoms TG, Smith RF, Cahn MD, Hartz TK (2012) Nitrogen requirements and N status determination of lettuce. HortScience 12:1768–1774

British Nutrition F (2009) Minerals and trace elements. Assessed on: March 06, 2020. Available at: https://www.nutrition.org.uk/nutritionscience/nutrients-food-and-ingredients/minerals-and-trace-elements.html?showall=1&limitstart

Britto DT, Kronzucker HJ (2002) NH4+ toxicity in higher plants: a critical review. J Plant Physiol 159:567–584

Bryson GM, Mills HA, Sasseville DN, et al (2014) Plant analysis handbook III. Athens, GA: Micro-Macro Publishing

Buchanan D, Omaye S (2013) Comparative Study of Ascorbic Acid and Tocopherol Concentrations in Hydroponic- and Soil-Grown Lettuces. Food Nutr Sci 10:1047–1053.

Cano A, Acosta M, Arnao MB (2003) Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (Lycopersicon esculentum Mill.). Postharvest Biol

Technol 28:59–65

Carr AC, Vissers MCM (2013) Synthetic or food-derived vitamin C—are they equally bioavailable? Nutrients 5:4284–4304

Chantigny M, Angers D, Belanger G, et al (2008) Yield and nutrient export of grain corn fertilized with raw and treated liquid swine manure. Agron J 1303–1309. https://doi.org/10.2134/agronj2007.0361

Chantigny MH, Angers DA, Rochette P, et al (2007) Gaseous Nitrogen Emissions and Forage Nitrogen Uptake on Soils Fertilized with Raw and Treated Swine Manure. J Environ Qual 36:1864–1872

Chapman HD, Pratt PF (1962) Methods of analysis for soils, plants and waters. Soil Sci 93:68

Cometti NN, Bremenkamp DM, Galon K, et al (2013) Cooling and concentration of nutrient solution in hydroponic lettuce crop. Hortic Bras 31:287–292

Conklin PL, Norris SR, Wheeler GL, et al (1999a) Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis. Proc Natl Acad Sci 96:4198–4203

Conklin PL, Norris SR, Wheeler GL, et al (1999b) Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis

Coronel G, Chang M, Rodríguez-Delfín A (2008) Nitrate reductase activity and chlorophyll content in lettuce plants grown hydroponically and organically. In International Symposium on Soilless Culture and Hydroponics 843. pp 137–144

Correia ECS (2013) Reação de cultivares de alface do grupo americano a Meloidoygine incognita, M. javanica e M. enterolobii (55f. Dissertação (Mestrado em Agronomia), Universidade Estadual Paulista “Júlio de Mesquita Filho”, Botucatu, Brazil).

Craig W, Beck L (1999) Phytochemicals: Health Protective Effects. Can J Diet Pract Res 60:78–

84

Cuevas-Rodri´guez EO, Rodri´guez R, Dia VP, et al (2010) Inhibition of Pro-inflammatory Responses and Antioxidant Capacity of Mexican Blackberry (Rubus spp.) Extracts. J Agric Food Chem 58:9542–9548. https://doi.org/10.1021/jf102590p

Dai J, Mumper RJ (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15:7313–7352

Davis JM, Murphy EA, Carmichael MD (2009) Effects of the dietary flavonoid quercetin upon performance and health. Curr Sport Med Rep 8:206–213

Dias JS, Ryder E (2011) World Vegetable Industry: Production, Breeding, Trends. Hort Rev 38:299–356

Drennan MF, DiStefano TD (2010) Characterization of the curing process from high-solids anaerobic digestion. Bioresour Technol 2:537–544

Duarte TL, Lunec J (2005) When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C. Free Radic Res 39:671–686

Durazzo A, Azzini E, Lazzé MC, et al (2014) Antioxidants in Italian head lettuce (lactuca sativa var. capitatal.) Grown in organic and conventional systems under greenhouse conditions. J Food Biochem 38:56–61

El-Shinawy MZ, Gawish SM (2006) Effect of commercial organic nutrient solutions on growth and chemical composition of lettuce under agricultural soilless system. Egypt J Hortic 33:19

Environmental Protection Agency (1979) Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020

Errebhi M, Wilcox GE (1990) Plant species response to ammonium-nitrate concentration ratios. J Plant Nutr 13:1017–1029

Esteban R, Ariz I, Cruz C, Moran J (2016) Review: Mechanisms of ammonium toxicity and the quest for tolerance. Plant Sci 248:92–101.

Fan J, Xiao J, Liu D, et al (2017) Effect of application of dairy manure, effluent and inorganic fertilizer on nitrogen leaching in clayey fluvo-aquic soil: A lysimeter study. Sci. Total Env 592:206–214

FAO (2018) The state of food insecurity in the world 2018. Available at: http://www.fao.org/state-of-food-security-nutrition. Assessed on: (April 20, 2020). Public Library of Science

Fernández-Cirelli A, Arumí JL, Rivera D, Boochs PW (2009) Environmental effects of irrigation in arid and semi-arid regions. Chil J Agric Res 27–40

Fishman M, Friedman L (1989) Methods for determination of inorganic substances in water and fluvial sediments. US Department of the Interior, Geological Survey.

Forge T, Kenney E, Hashimoto N, et al (2016) Compost and poultry manure as preplant soil amendments for red raspberry: Comparative effects on root lesion nematodes, soil quality and risk of nitrate leaching. Agric Ecosyst Environ 223:48–58

Fullbloom (2020) Hydroponic Systems 101 | Learn The Basics of Hydroponics. Available at: https://www.fullbloomgreenhouse.com/hydroponic-systems-101/ (Assessed on July 05, 2020).

Furukawa Y, Hasegawa H (2006) Response of Spinach and Komatsuna to Biogas Effluent Made from Source-Separated Kitchen Garbage. J Environ Qual 35:1939–1947

Gagliardini E, Buelli S, Benigni A (2011) Endothelin in chronic proteinuric kidney disease. Contrib Nephrol 172:171-184.

Garbin ML, Dillenburg LR (2008) Effects of different nitrogen sources on growth, chlorophyll

concentration, nitrate reductase activity and carbon and nitrogen distribution in Araucaria angustifolia. Brazilian J Plant Physiol 20:295–303

Gavrilova V, Kajd M, Gjamovski V, Stefova M (2011) Separation, Characterization and Quantification of Phenolic Compounds in Blueberries and Red and Black Currants by HPLCÀDADÀESI-MS n. J Agric Food Chem 59:4009–4018

Gell K, van Groenigen J, Cayuela ML (2011) Residues of bioenergy production chains as soil amendments: immediate and temporal phytotoxicity. J Hazard Mater 186:2017–2025

Georgacakis D, Sievers DM, Iannotti EL (1982) Buffer stability in manure digesters. Agric Wastes 4:427–441

Gerendás J, Zhu Z, Bendixen R, et al (1997) Physiological and biochemical processes related to ammonium toxicity in higher plants. J Plant Nutr Soil Sci 160:239–251

Gliszczyńska-Świgło A (2007) Folates as antioxidants. Food Chem 101:1480–1483

Goddek S, Schmautz Z, Scott B, et al (2016) The effect of anaerobic and aerobic fish sludge supernatant on hydroponic lettuce. Agronomy 2:37

Gómez X, Cuetos MJ, García AI, Morán A (2007) An evaluation of stability by thermogravimetric analysis of digestate obtained from different biowastes. J Hazard Mater 149:97–105

Gong W, Yan X, Wang J, et al (2011) Long-term applications of chemical and organic fertilizers on plant-available nitrogen pools and nitrogen management index. Biol Fertil Soils 47:767–775

Gupta UC, Gupta SC (2014) Sources and deficiency diseases of mineral nutrients in human health and nutrition: a review. Pedosphere 24:13–38

Hao XJ, Hong JP, Gao WJ (2007) Effect of biogas slurry and biogas residues on quality of

greenhouse mini cucumber. [J] Soil Fertil Sci China 5:9

Hariadi YC, Nurhayati AY, Hariyani P (2016) Biophysical monitoring on the effect on different composition of goat and cow manure on the growth response of maize to support sustainability. Agric Agric Sci Procedia 118–127

Harris E (2001) Copper homeostasis: the role of cellular transporters. Nutr Rev 59:281–285

Hassan SA, Mijin S, Yusoff UK, et al (2012) Nitrate, ascorbic acid, mineral and antioxidant activities of Cosmos caudatus in response to organic and mineral-based fertilizer rates. Molecules 17:7843–7853

Haukioja E, Ossipov V, Koricheva J, et al (1998) Biosynthetic origin of carbon-based secondary compounds: cause of variable responses of woody plants to fertilization? Chemoecology 8:133–139

Healy EF, Sanders J, King PJ, Edwards Robinson WJ (2009a) A docking study of L-chicoric acid with HIV-1 integrase. J Mol Graph Model 27:584–589

Healy EF, Sanders J, King PJ, Robinson Jr WE (2009b) A docking study of L-chicoric acid with HIV-1 integrase. J Mol Graph Model 27:584–589

Heimler D, Isolani L, Vignolini P, Romani A (2009) Polyphenol content and antiradical activity of Cichorium intybus L. from biodynamic and conventional farming. Food Chem 114:765–770

Herrera E, Jiménez R, Aruoma OI, et al (2009) Aspects of Antioxidant Foods and Supplements in Health and Disease. Nutr Rev 67:140–144

Hjorth M, Christensen K V., Christensen ML, Sommer SG (2010) Solid—liquid separation of animal slurry in theory and practice. A review. Agron Sustain Dev 30:153–180

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil.

Circular. California agricultural experiment station, 347(2nd edit).

Hobson P, Wheatley A (1992) Anaerobic digestion – modern theory and practice. Elsevier Appl Sci 269

Hokkaido (2003) Research report of environment, resources, and recycling in Hokkaido, Sapporo. Civil Engineering Institute of Hokkaido, Sapporo (in Japanese).

Hoque MM, Ajwa HA, Smith R (2007) Nitrite and ammonium toxicity on lettuce grown under hydroponics. Commun Soil Sci Plant Anal 39:207–216

Hord NG, Tang Y, Bryan NS (2009a) Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 90:1-10.

Hord NG, Tang Y, Bryan NS (2009b) Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 90:1–10

Hung HC, Joshipura KJ, Jiang R, et al (2004) Fruit and vegetable intake and risk of major chronic disease. J Natl Cancer Inst 96:1577–1584

Ibrahim MH, Jaafar HZ, Karimi E, Ghasemzadeh A (2013) Impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kacip Fatimah (Labisia pumila Benth). Molecules 18:10973–10988

Insam H, Gómez-Brandón M, Ascher J (2015) Manure-based biogas fermentation residues– Friend or foe of soil fertility? Soil Biol Biochem 84:1–14

IPL (2020) Hydroponics Advantages And Disadvantages. Available at: https://www.ipl.org/essay/Hydroponics-Advantages-And-Disadvantages-PKNBD2NFCEDR (Assessed on July 05, 2020).

Joshi R, Adhikari S, Patro BS, et al (2001) Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. Free Radic Biol Med 30:1390–1399

Kapoulas N, Koukounaras A, Ilić ZS (2017) Nutritional quality of lettuce and onion as companion plants from organic and conventional production in north Greece. ElsevierScientia Hortic 219:310–318

Karakaya S (2004) Bioavailability of Phenolic Compounds. Crit Rev Food Sci Nutr 44:453–464

Kempinski CF, Haffar R, Barth C (2011) Toward the mechanism of NH4+ sensitivity mediated by Arabidopsis GDP-mannose pyrophosphorylase. Plant, Cell Environ 34:847–858

Kim MJ, Moon Y, Tou JC, et al (2016) Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). J Food Compos Anal 49:19–34

Kirchmann H, Bernal M (1997) Organic waste treatment and C stabilization efficiency. Soil Biol Biochem 29:1747–1753

Klouberta V, Rink. L (2015) Zinc as a micronutrient and its preventive role of oxidative damage in cells. Food Funct 6:3195–3204

Knapp Haraldsen T, Andersen U, Krogstad T, Sørheim R (2011) Liquid digestate from anaerobic treatment of source-separated household waste as fertilizer to barley. Waste Manag Res 29:1271–1276

Koester RP, Skoneczka JA, Cary TR, et al (2014) Historical gains in soybean (Glycine max Merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. J Exp Bot 12:3311–3321

Kris-Etherton PM, Hecker KD, Bonanome A, et al (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 9:71–88

Krupa S V. (2003) Effects of atmospheric ammonia (NH3) on terrestrial vegetation: a review. Envionmental Pollut 124:179–221

Lafleur B, Thiffault E, Paré D, et al (2012) Effects of hog manure application on the nutrition

and growth of hybrid poplar (Populus spp.) and on soil solution chemistry in short-rotation woody crops. Agric Ecosyst environmen 155:95–104

Lam V, Watanabe T, Phan T, Khai L (2002) A case study: introduction of low-cost biogas digester to small-scale farming systems

Lampkin N (1990) Organic farming. Farming press books. Ipswich. United Kingdom.

Li B, Li G, Kronzucker HJ, et al (2014) Ammonium stress in Arabidopsis: signaling, genetic loci, and physiological targets. Trends Plant Sci 19:107–114

Li B, Li Q, Xiong L, et al (2012) Arabidopsis plastid AMOS1/EGY1 integrates abscisic acid signaling to regulate global gene expression response to ammonium stress. Plant Physiol 160:2040–2051

Li BH, Shi. WM (2007) Effects of elevated NH4+ on Arabidopsis seedlings different in accessions. Acta Pedofil Sin 44:508–515

Li Q, Li X, Tang B, Gu M (2018) Growth responses and root characteristics of lettuce grown in aeroponics, hydroponics, and substrate culture. Horticulturae 4:35

Liedl BE, Cummins M, Young A, et al (2004) Hydroponic lettuce production using liquid effluent from poultry waste bioremediation as a nutrient source. In VII International Symposium on Protected Cultivation in Mild Winter Climates: Production, Pest Management and Global Competition 659. pp 721–728

Liedl BE, Wilfong K, Taylor C, Mazzaferro K (2006) Liquid effluent from poultry waste bioremediation as a nutrient source for hydroponic cucumber production. HortScience 4:997A – 997

Lin D, Xiao M, Zhao J, et al (2016) An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules 21:1374

Lind OP, Hultberg M, Bergstrand KJ, et al (2020) Biogas Digestate in Vegetable Hydroponic Production: pH Dynamics and pH Management by Controlled Nitrification. Waste and Biomass Valorization 1–11

Liu WK, Yang QC, Du L (2009) Soilless cultivation for high-quality vegetables with biogas manure in China: Feasibility and benefit analysis. Renewable Agriculture and Food Systems,. Renew Agric Food Syst 300–307

Liu WK, Yang QC, du Lian F, et al (2011) Nutrient supplementation increased growth and nitrate concentration of lettuce cultivated hydroponically with biogas slurry. Acta Agric Scand Sect B Soil Plant Sci 61:391–394

Liu X, Ardo S, Bunning M, et al (2007) Total phenolic content and DPPH radical scavenging activity of lettuce (Lactuca sativa L.) grown in Colorado. LWT Food Sci Technol 40:552–557

Liu Y, Lai N, Gao K, et al (2013) Ammonium inhibits primary root growth by reducing the length of meristem and elongation zone and decreasing elemental expansion rate in the root apex in Arabidopsis thaliana. PLoS One 8:e61031.

Liu YJ, Tong YP, Zhu YG, et al (2006) Leaf chlorophyll readings as an indicator for spinach yield and nutritional quality with different nitrogen fertilizer applications. J Plant Nutr 29:1207–1217

Llorach R, Martínez-Sánchez A, Tomás-Barberán FA, et al (2008) Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem 108:1028–1038

Loqué D, Mora SI, Andrade SL, et al (2009) Pore mutations in ammonium transporter AMT1 with increased electrogenic ammonium transport activity. J Biol Chem 284:24988–24995

Loria ER, Sawyer JE, Barker DW, et al (2007) Use of anaerobically digested swine manure as a nitrogen source in corn production. In: Agronomy Journal. pp 1119–1129

Madeira AC, De Varennes A (2005) Use of chlorophyll meter to assess the effect of nitrogen on sweet pepper development and growth. J Plant Nutr 28:1133–1144

Makádi M, Orosz A, Tomócsik V (2012) Digestate: A New Nutrient Source - Review, Biogas, Dr. Sunil Kumar (Ed.), ISBN: 978-953-51-0204-5, InTech, Available from: http://www.intechopen.com/books/biogas/digestate-a-new-nutrient-source-review

Makádi M, Tomócsik A, Kátai J, et al (2008) Nutrient cycling by using residues of bioenergy production - effects of biogas-digestate on plant and soil parameters. Cereal Res Commun 1807–1810

Mampholo BM, Maboko MM, Soundy P, Sivakumar D (2016) Phytochemicals and Overall Quality of Leafy Lettuce (Lactuca sativa L.) Varieties Grown in Closed Hydroponic System. J Food Qual 39:805–815

Man J, Shi Y, Yu Z, Zhang Y (2015) Dry matter production, photosynthesis of flag leaves and water use in winter wheat are affected by supplemental irrigation in the Huang-Huai-Hai Plain of China. PLoS One 10:e0137274.

Mapson LW, Cruickshank EM (1947) Effect of various salts on the synthesis of ascorbic acid and carotene in cress seedlings. Biochem J 41:197-205.

Masarirambi MT, Hlawe MM, Oseni OT, Sibiya TE (2010) Effects of organic fertilizers on growth, yield, quality and sensory evaluation of red lettuce (Lactuca sativa L.) ‘Veneza Roxa.’ Agric Biol J N Am, 1:1319–1324

Massé DI, Talbot G, Gilbert Y (2011) On farm biogas production: A method to reduce GHG emissions and develop more sustainable livestock operations. Anim Feed Sci Technol 436–

445

Mayer-Foulkes DA (2011) A survey of macro damages from non-communicable chronic diseases: Another challenge for global governance. Glob Econ J 11:1–26

Meagy MJ, Eaton TE, Barker A V. (2016) Assessment of mineral nutrient concentrations of lettuce in response to cultivar selection and fertilization in greenhouse production. J Plant Nutr 39:1796–1808

Medicine I of (2005) Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Institute of Medicine, Washington D.C. National Academies Press (US).

Medicine I of (1997) Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Institute of Medicine, Washington, D.C. National Academies Press (US).

Medicine I of (1998) Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Institute of Medicine, Washington, D.C. National Academies Press (US).

Medina E (1984) Nutrient balance and physiological processes at the leaf level. In Physiological ecology of plants of the wet tropics (pp. 139-154). Springer, Dordrecht. pp 139–154

Menardo S, Gioelli F, Balsari P (2011) The methane yield of digestate: effect of organic loading rate, hydraulic retention time, and plant feeding. Bioresour Technol 102:2348–2351

Mihaljević S, Radić S, Bauer N, et al (2011) Ammonium-related metabolic changes affect somatic embryogenesis in pumpkin (Cucurbita pepo L.). J Plant Physiol 168:1943–1951

Miller J, Rucker R (2012) Pantothenic acid. In: Erdman JW, Macdonald IA, Zeisel SH, eds. Present Knowledge in Nutrition. 10th ed. Washington, DC: Wiley-Blackwell:375-90.

Mohankumar JB, Uthira L, Maheswari SU (2018) Total phenolic content of organic and conventional green leafy vegetables. J Nutr Hum Heal 2:1–6

Mollavali M, Perner H, Rohn S, et al (2018) Nitrogen form and mycorrhizal inoculation amount and timing affect flavonol biosynthesis in onion (Allium cepa L.). Mycorrhiza 28:59–70

Möller K, Schulz R, Müller T (2010) Substrate inputs, nutrient flows and nitrogen loss of two centralized biogas plants in southern Germany. Nutr Cycl Agroecosystems 87:307–325

Möller K, Stinner W, Deuker A, Leithold G (2008a) Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. Nutr Cycl Agroecosystems 82:209–232

Möller K, Stinner W, Deuker A, Leithold G (2008b) Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. Nutr Cycl Agroecosystems 82:209–232

Morris MC, Evans DA, Tangney CC, et al (2006) Associations of vegetable and fruit consumption with age-related cognitive change. Neurology 67:1370–1376

Mou B (2009) Nutrient content of lettuce and its improvement. Curr Nutr Food Sci 5:242-248.

Mou B, Ryder EJ (2004) Relationship between the nutritional value and the head structure of lettuce. Acta Hortic 637:361–7

Mulla D, Birr A, Randall G, et al (2001) Impacts of animal agriculture on water quality: technical work paper. Minnesota Environmental Quality Board, Minnesota Planning (Agency), St. Paul.

Nicola S, Hoeberechts J, Fontana E (2005) Comparison between traditional and soilless culture systems to produce rocket (Eruca sativa) with low nitrate content. In International Symposium on Soilless Culture and Hydroponics; International Society for. pp 549–555.

Nicoletto C, Santagata S, Zanin G, Sambo P (2014) Effect of the anaerobic digestion residues use on lettuce yield and quality. Sci Hortic (Amsterdam) 180:207–213

Nicolle C, Carnat A, Fraisse D, et al (2004) Characterisation and variation of antioxidant micronutrients in lettuce (Lactuca sativa folium). J Sci Food Agric 84:2061–2069

Nkoa R (2014) Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. Agron Sustain Dev 34:473-492.

Norenberg MD, Rama Rao K V., Jayakumar AR (2009) Signaling factors in the mechanism of ammonia neurotoxicity. Metab. Brain Dis. 24:103–117

Olsen KM, Lea US, Slimestad R, et al (2008) Differential expression of four Arabidopsis PAL genes; PAL1 and PAL2 have functional specialization in abiotic environmental-triggered flavonoid synthesis. J Plant Physiol 165:1491–1499

Paavola T, Rintala J (2008) Effects of storage on characteristics and hygienic quality of digestates from four co-digestion concepts of manure and biowaste. Bioresour Technol 99:7041–7050

Pan Z, Qi G, Andriamanohiarisoamanana FJ, et al (2018) Potential of anaerobic digestate of dairy manure in suppressing soil-borne plant disease. Anim Sci J 89:1512–1518

Pavia M, Pileggi C, Nobile CGA, Angelillo IF (2006) Association between fruit and vegetable consumption and oral cancer: a meta-analysis of observational studies. Am J Clin Nutr 83:1126–1134

Pellati F, Benvenuti S, Magro L, et al (2004) Analysis of phenolic compounds and radical scavenging activity of Echinacea spp. J Pharm Biomed Anal 35:289–301

Pérez-Jiménez J, Neveu V, Vos F, Scalbert A (2010) Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. Eur J Clin Nutr 64:112–120

Petropoulos SA, Chatzieustratiou E, Constantopoulou E, Kapotis G (2016) Yield and quality of

lettuce and rocket grown in floating culture system. Not Bot Horti Agrobot Cluj-Napoca 44:603–612

Pognani M, D'Imporzano G, Scaglia B, Adani F (2009) Substituting energy crops with organic fraction of municipal solid waste for biogas production at farm level: A full-scale plant study. Process Biochem 44:817–821

Przygocka-Cyna K, Grzebisz W, Lukowiak R, Biber M (2018) Biomass ash and biogas digestate bio-fertilizers as a source of nutrients for light acid soil–an exhaustion test. J Elem 23:413–428

Qi X, Zhang S, Wang Y, Wang R (2005) Advantages of the integrated pig-biogas-vegetable greenhouse system in North China. Ecol Eng 24:175–183

Qin C, Qian W, Wang W, et al (2008) GDP-mannose pyrophosphorylase is a genetic determinant of ammonium sensitivity in Arabidopsis thaliana

Qu H, Madl RL, Takemoto DJ, et al (2005) Lignans are involved in the antitumor activity of wheat bran in colon cancer SW480 cells. J Nutr 3:598–602

Rajashekar CB, Oh M-M, Carey EE (2012) Organic Crop Management Enhances Chicoric Acid Content in Lettuce. Food Nutr Sci 3:1296–1302

Rezk BM, Haenen GR, van der Vijgh WJ, Bast A (2003) Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophores. FEBS Letters. Elsevier 555:601– 605

Roberts S (2018) What Is the Main Function of Phosphorus in the Body? Assessed on: March 06, 2020. Available at: https://healthyeating.sfgate.com/main-function-phosphorus-body-5789.html

Ronga D, Setti L, Salvarani C, et al (2019) Effects of solid and liquid digestate for hydroponic

baby leaf lettuce (Lactuca sativa L.) cultivation. Sci Hortic (Amsterdam) 172–181

Roosta HR, Schjoerring JK (2007) Effects of ammonium toxicity on nitrogen metabolism and elemental profile of cucumber plants. J Plant Nutr 30:1933–1951

Sacks FM, Willett WC, Smith A, et al (1998) Effect on Blood Pressure of Potassium, Calcium, and Magnesium in Women With Low Habitual Intake. Hypertension 31:131–138

Salminen E, Rintala J, Härkönen J, et al (2001) Anaerobically digested poultry slaughterhouse wastes as fertiliser in agriculture. Bioresour Technol 78:81–88

Santamaria P (2006) Nitrate in vegetables: Toxicity, content, intake and EC regulation. J. Sci. Food Agric. 86:10–17

Santos FT, Goufo P, Santos C, et al (2016) Comparison of five agro-industrial waste-based composts as growing media for lettuce: Effect on yield, phenolic compounds and vitamin C. Food Chem 209:293–301

Sapkota S, Sapkota S, Liu Z (2019) Effects of nutrient composition and lettuce cultivar on crop production in hydroponic culture. Horticulturae 5:72

Savvas D, Passam HC, Olympios C, et al (2006) Effects of Ammonium Nitrogen on Lettuce Grown on Pumice in a Closed Hydroponic System

Scaglia B, Pognani M, Adani F (2017) The anaerobic digestion process capability to produce biostimulant: the case study of the dissolved organic matter (DOM) vs. auxin-like property. Sci Total Environ 589:36-45.

Scott J, Rébeillé F, Fletcher J (2000) Folic acid and folates: The feasibility for nutritional enhancement in plant foods. J. Sci. Food Agric. 80:795–824

Seefeldt S (2015) Crop Production and Soil Mangement Series Steven Seefeldt, Extension Faculty, Agriculture and Horticulture. Assessed on: September 14, 2020. Available at:

http://cespubs.uaf.edu/index.php/download_file/1025/.

Shohag MJI, Wei Y, Yang X (2012) Changes of Folate and Other Potential Health-Promoting Phytochemicals in Legume Seeds As Affected by Germination. J Agric Food Chem 60:9137–9143

Silber A, Bar-Tal A (2008) Nutrition of substrate-grown plants. Soil Cult Theory Pract Elsevier, San Diego, CA, 4:291-339.

Slamet W, Purbajanti ED, Darmawati A, Fuskhah E (2017) Leaf area index, chlorophyll, photosynthesis rate of lettuce (Lactuca sativa L) under N-organic fertilizer. Indian J Agric Res 4:365–369

Soetan KO, Olaiya CO, Oyewole OE (2010) The importance of mineral elements for humans, domestic animals and plants: A review. African J food Sci 5:200-222.

Somasundaram E, Amanullah MM, Vaiyapuri K, et al (2007) Influence of organic sources of nutrients on the yield and economics of crops under maize based cropping system. J Appl Sci Res 1774-1777.

Sommer SG, Husted S (1995a) A simple model of pH in slurry. J Agric Sci 124:447–453

Sommer SG, Husted S (1995b) The chemical buffer system in raw and digested animal slurry

Southon S (2000) “Increased Fruit and Vegetable Consumption within the EU: Potential Health Benefits,” Food Research International. 33:211-217.

Squire GR, Ong CK, Monteith JL (1987) Crop growth in semi-arid environment. In: Proceedings of 7th International Workshop, 7-11 April, 1986, International Crops Research Institute for Semi-Arid Tropics. Patancheru, Hyderabad, pp 219-231.

Stinner W, Möller K, Leithold G (2008) Effects of biogas digestion of clover/grass-leys, cover crops and crop residues on nitrogen cycle and crop yield in organic stockless farming

systems. Eur J Agron 29:125–134

Stoknes K, Scholwin F, Krzesiński W, et al (2016) Efficiency of a novel “Food to waste to food” system including anaerobic digestion of food waste and cultivation of vegetables on digestate in a bubble-insulated greenhouse. Waste Manag 466–476

Su YY, Lu Y, Shi WS (2008) Effect of biogas fertilizer on yield and quality of lettuce in soilless culture. [J] Soil Fertil Sci China 1

Sulaiman SF, Yusoff NAM, Eldeen IM, et al (2011) Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (Musa sp.). J Food Compos Anal 1–10.

Survase SA, Bajaj IB, Singhal RS (2006) Biotechnological production of vitamins. Food Technol Biotechnol 44:381–396

Szűcs B, Simon M, Füleky G (2006) Anaerobic pre-treatment effects on the aerobic degradability of wastewater sludge. Proceedings of the Internationale Conference ORBIT 2006, Weimar, 13-15 September, 2006. Part 2, pp. pp 425-434.

Tambone F, Genevini P, D’Imporzano G, Adani F (2009) Assessing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW. Bioresour Technol 100:3140–3142

Tambone F, Scaglia B, D’Imporzano G, et al (2010) Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 5:577–583

Tamilselvi SM, Chinnadurai C, Ilamurugu K, et al (2015) Effect of long-term nutrient managements on biological and biochemical properties of semi-arid tropical Alfisol during

maize crop development stages. Ecol Indic 48:76–87

Tani M, Sakamoto N, Kishomoto T, Umetsu K (2006) Utilization of anaerobically digested slurry combined with other waste following application to agricultural land. Int Congr Ser 1293:331.

Teglia C, Tremier A, Martel JL (2011) Characterization of solid digestates: Part 2, assessment of the quality and suitability for composting of six digested products. Waste and Biomass Valorization 2:113–126

Tian X, Li S, Wang Z, et al (2003) Response of lettuce to different nitrogen forms. Ying yong sheng tai xue bao=. J Appl Ecol 14:377

Tiquia SM, Tam NFY, Hodgkiss IJ (1996) Effects of composting on phytotoxicity of spent pig-manure sawdust litter. Environ Pollut 93:249–256

Tiwari VN, Tiwari KN, Upadhyay RM (2000) Effect of crop residues and biogas slurry incorporation in wheat on yield and soil fertility. J Indian Soc Soil Sci 48:515–520

Trumbo P (2014) Pantothenic acid. In: Ross AC, Caballero B, Cousins RJ, et al., eds. Modern Nutrition in Health and Disease. 11th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2014:351-7.

Tshikalange B, Bello ZA, Ololade OO (2020) Comparative nutrient leaching capability of cattle dung biogas digestate and inorganic fertilizer under spinach cropping condition. Environ Sci Pollut Res 27:3237–3246

USDA (2015) National Nutrient Database for Standard Reference Release 28. USDA, Washington, D.C.

Valverde-Barrantes OJ, Freschet GT, Roumet C, Blackwood CB (2017) A worldview of root traits: the influence of ancestry, growth form, climate and mycorrhizal association on the

functional trait variation of fine-root tissues in seed plants. New Phytol 215:1562–1573

Wargovich MJ (2000) Anticancer Properties of Fruits and Vegetables

Warman PR, Havard KA (1997) Yield, vitamin and mineral contents of organically and conventionally grown carrots and cabbage. Agric Ecosyst Environ 61:155–162

Watson SA, Noggle. GR (1947) Effect of mineral deficiencies upon the synthesis of riboflavin and ascorbic acid by the oat plant. Plant Physiol 22:228–243

Webb AR, Hawkes FR (1985) Laboratory scale anaerobic digestion of poultry litter: Gas yield-loading rate relationships. Agric wastes 13:31–49

Weiland P (2010) Biogas production: current state and perspectives. Appl Microbiol Biotechnol 849–860

Wenke L, Du L, Yang Q (2009) Biogas slurry added amino acids decreased nitrate concentrations of lettuce in sand culture. Acta Agric Scand Sect B Soil Plant Sci 59:260–264

Wright AF, Bailey JS (2001) Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 dry combustion analyzer. Commun Soil Sci Plant Anal 32:3243–3258

Xu WH, Wang ZY, Quan YM, et al (2003a) Effect of application of biogas slurry on nitrate content and nutrition quality of lettuce and romaine lettuce. Rural Eco-Environment, 19:34–37

Xu WH, Wang ZY, Wang Q, et al (2003b) Effects of different biogas slurry on nutrient quality and nitrate content of lettuce. China biogas 21:11–13

Yalovsky S, Bloch D, Sorek N, Kost B (2008) Regulation of membrane trafficking, cytoskeleton dynamics, and cell polarity by ROP/RAC GTPases. Plant Physiol 147:1527–1543

You Q, Chen F, Wang X, et al (2012) Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. LWT-Food Sci Technol 46:164–168

Yu FB, Luo XP, Song CF, et al (2010) Concentrated biogas slurry enhanced soil fertility and tomato quality. Acta Agric Scand Sect B Soil Plant Sci 60:262–268

Zandvakili OR, Barker A V., Hashemi M, et al (2019) Comparisons of commercial organic and chemical fertilizer solutions on growth and composition of lettuce. J Plant Nutr 42:990–1000

Zandvakili OR, Ebrahimi E, Hashemi M, et al (2017) The potential of green manure mixtures to provide nutrients to a subsequent lettuce crop. Commun Soil Sci Plant Anal 48:2246–2255

Zhao X, Carey EE, Young JE, et al (2007) Influences of organic fertilization, high tunnel environment, and postharvest storage on phenolic compounds in lettuce. HortScience 42:71–76

Zuo J, Tang W, Xu Y (2015) Anti-Hepatitis B Virus Activity of Chlorogenic Acid and Its Related Compounds. In Coffee in health and disease prevention (pp. 607-613). Academic Press.

Chapter 4

4. General Discussion and Conclusion

The objectives of the present study were:

I. To evaluate the effects of DD, standard nutrient solution (NS) and their combined application (50% DD + 50% NS) on growth and yield of hydroponically grown lettuce.

II. To determine the effect of DD, NS and combination of NS+DD on minerals and vitamins in hydroponically grown lettuce.

III. To investigate the effects of DD, NS alone and combination of both nutrient solutions on bioactive compounds (phenolic content, flavonoids, total antioxidants) in hydroponically grown lettuce in greenhouse setting.

These objectives were accomplished through two separate experiments as described in Chapter 2 and 3. Effects of anaerobic dairy digestate on growth and yield of lettuce in a hydroponic greenhouse production system - a pilot scale study - is described in Chapter 2. Whereas, influence of dairy digestate on minerals, vitamins and phytochemical profile of hydroponically grown lettuce varieties is given in Chapter 3.

4.1. Effect of DD and inorganic nutrient solution on plant growth, yield and nitrogen uptake (NO_3^--N, NH_4^+-N) of lettuce

Plant growth is most often restricted due to non-availability of resources, predominantly water and essential nutrients in the growing medium in addition to optimum growing conditions. Leaf area is a vital parameter that determines light interception (Koester et al. 2014) and contribute to the photosynthesis which leads to improving the crop yield (Man et al. 2015). Mineral nutrients either in soil solution or hydroponics play an important role in enhancing LA of lettuce. For example, Medina (1984) observed that nitrogen and phosphorus played a significant role in enhancing LA and consequently increased photosynthesis which is due to readily availability of mineral nutrients in NS, specially N that enhanced LA (Hariadi et al. 2016). In a previous study, Zandvakili et al. (2019) reported higher LA of lettuce grown with inorganic fertilizers compared to lower LA in liquid organic solution which confirms the finding of present study.

Plants use sunlight energy, CO_2 and water to produce sugars with the involevement of chlorophyll during photosynthesis process. N is integral part of chlorophyll, as it is of amino acids, which are the building blocks of proteins. and of energy transfer compounds such as ATP (adenosine triphosphate) which is required for the proper functioning of plants and subsequent plant growth. Various studies showed that inorganic nutrient solution which predominantly contains higher NO_3^--N resulted in higher chlorophyll content in lettuce (Madeira and De Varennes 2005; Zandvakili et al. 2019) which could be attributed to consistent and optimum supply of N as well as other essential nutrients whereas, lower NO_3^--N in DD solution resulted in reduced leaf chlorophyll content (Table 2).

Plants that are grown in predominant NO_3^--N solution displayed higher root growth, root mass and highly branched roots system than higher NH_4^+-N fed plants (Garbin and Dillenburg 2008).

Ammonium toxicity causes root and shoot growth inhibition which is linked with leaf discoloration, ionic imbalance and various oxidative stresses (Bittsánszky et al. 2015; Esteban et al. 2016). At the cellular level, NH_4^+-N is an essential substrate for the production of amino acid and protein in all living organisms, but it is devastating to the cells when present in excess (Norenberg et al. 2009). In previous studies similar results such as stunted root growth which is a key phenotypic indicator of toxicity were reported where plants were subjected to high NH_4^+-N concentration in root zone (Britto and Kronzucker 2002; Li et al. 2014). A study on *Arabidopsis thaliana* conducted by Liu et al. (2013) revealed the stunted and reduced root growth due to higher NH_4^+-N in nutrient solution which possibly obstructed the meristem cells during the mitosis which abridged the root growth. In the present study, we also observed stunted root growth and the lowest root dry weight due to higher NH_4^+-N in DD and NS+DD treatments compared to NS treatment.

Crop yield relies on numerous factors which include crop genotypes or species, soil fertility or mineral nutrient composition and environmental variables (Sapkota et al. 2019). In present study, the Romaine cultivar grown in NS showed superior performance in term of yield, whereas, the Newham variety cultivated in DD treatment produced lowest yield. Higher yield of Romaine cultivated in NS treatment could be attributed to an optimum NO_3^-:NH_4^+, and consistent supply of other essential nutrients and genetic potential of Romaine, contrary to the lowest yield of Newham grown in DD treatment solution. Our results are in line with the findings of Zandvakili et al. (2019) and Barker et al. (2017), where authors reported lower yields of lettuce and cabbage grown with organic fertilizers than chemical fertilizer, respectively. Furthermore, several species showed varied preferences to N sources, for example, some species preferred NH_4^+ as N sources (Britto and Kronzucker 2002), tomato and cucumber prefer NO_3^- as N source (Roosta and

Schjoerring 2007), some species prefer combined application of both N sources (NO_3^- and NH_4^+) over the sole source (Errebhi and Wilcox 1990).

Root hairs are integral part of plant main root system which increase the surface area and help to uptake the minerals from surroundings. Excessive NH_4^+ concentration in the solution triggers the overexpression of Rho-related GTPase of Plants (ROP) via NADPH oxidase-mediated reactive oxygen species (ROS) formation (Yalovsky et al. 2008), which leads to swelling of root hairs (Bloch et al. 2011), ultimately reduced the mineral uptake and leads towards reduction in the yield. LA and chlorophyll content have a great contribution towards the yield. Optimum LA intercept higher light and more chlorophyll content produced more ATP and ultimately produced higher yield. Higher LA and chlorophyll content of lettuce enhanced yield when cultivated in NS treatment (Zandvakili et al. 2019). Loqué et al. (2009) reported that the gene AMOS1/EGY1 (Plastid metalloprotease), that synthesizes chlorophyll content, dependents on plastid signaling pathway, which is required for the expression of NH_4^+ responsive genes and the maintenance of chloroplast functionality. Chloroplast is directly linked with plant photosynthesis process. Under NH_4^+ stress, the chloroplast receives the stress signal (the plasma membrane acting as the first site of perception of NH_4^+ stress), and triggering of AMOS1/EGY1-dependents retrograde signaling and recruiting downstream abscisic acid (ABA) signaling, to regulate the expression of NH_4^+ which possibly affect the plant growth perticulary shoot and ultimately the yield (Li et al. 2012). High NH_4^+- N in DD and NS+DD solution treatments in the present study significantly affected root growth and resulted the lowest LA, chlorophyll content and the final yield.

4.2. Effect of DD and inorganic nutrient solution on mineral, vitamins and phytochemical contents of lettuce

Leafy vegetables are an abundant source of minerals obligatory for human health, but, the concentration of these minerals depends on nutrients availability from soil solution or any other substrate (Silber and Bar-Tal 2008). For instance, Hassan et al. (2012) reported lower mineral concentration in organically grown *Cosmos caudatus* than inorganic nutrient solution, possibly due to slow release of nutrients from organic solution/source. In present study, we also observed lower levels of minerals (K^+, Ca^{2+} and Mg^{2+}) in lettuce cultivated in DD solution compared to higher minerals in NS. Higher concentration of NH_4^+- N and elevated pH in DD may cause ionic imbalance and lessen the uptake of essential cations such as Ca^{2+}, K^+ and Mg^{2+} (Barker et al. 1967; Gerendás et al. 1997; Roosta and Schjoerring 2007). Zandvakili et al. (2019) investigated the effects of organic and inorganic nutrient solutions on four lettuce cultivars, and observed the lower concentration of N, K, and Ca in organically grown lettuce than inorganically fed lettuce. They further observed disparity in minerals among different cultivars, for instance, Arroyo cultivar displayed higher minerals uptake than other lettuce cultivars which might be due to difference in head morphology, smiliar trend was also observed by Mou and Ryder (2004). Zandvakili et al. (2019) reported that lettuce with loose head morphology tends to uptake more minerals than tighter head. We also observed higher concentration of minerals in Romaine than Newham in present study, which could be due to loose head morphology. However, further investigation is required to determine the phenomenon of higher and lower mineral uptake in loose and tighter head morphology in hydroponics with organic or inorganic nutrient supply. Contrary to that Warman and Havard (1997) and Kapoulas et al. (2017) reported the higher Mn and Zn uptake in organically grown cabbage and lettuce than conventionally grown lettuce. Whereas, a study conducted by

Zandvakili et al. (2019) observed the lower Zn and higher Mn concentration in inorganically grown lettuce than organically fed lettuce. However, in present study we observed lower concentration of Mn, Zn and B in lettuce leaves when grown in DD. Higher NH_4^+-N in nutrient solution tends to increase the solution pH which might have reduced the availability of Mn, Cu and Zn in the lettuce leaves (Savvas et al. 2006).

Increased solution pH from 6.0 to 7.0 decreased solubility and uptake of Mn and Zn in plants (Adams 2002). Lower concentration of these micronutrients in lettuce grown in DD in present study could be attributed to lower mineral nutrient composition and higher pH of DD (Figure 14), that might have reduced solubility resulted in low uptake of these micronutrients.

Crude protein concentration and total N contents in the leaves are directly linked to each other. Presence of high NO_3^- in the NS might have increased N uptake in plants and ultimately crude protein (Bryson et al. 2014). In present study, NS treatment increased 12% and 25% N uptake due to immediate availability of N and other nutrients than NS+DD and DD treatments and consequently higher crude protein. Our results are in agreement with that reported by Zandvakili et al., (2019), who observed high crude protein in inorganically grown lettuce compared to organically fed plants.

Vitamins refer to naturally occurring organic compounds in leafy vegetables are important for proper metabolism (Survase et al. 2006) and an important medical therapeutic agents (Kim et al. 2016). Previous studies reported biogas slurry increased the vitamin C contents in cucumber (Hao et al. 2007), bok choi (Liu et al. 2006) and in lettuce (Su et al. 2008). Whereas, Xu et al. (2003a, b) observed lower contents of vitamin C in lettuce either grown in organic fertilizer or in agro-industrial waste compost and higher vitamin C with inorganic N fertilized lettuce. In present study, we observed lower vitamin C in DD grown lettuce which might be due to higher NH_4^+-N in solution and diminished vitamin C production (Mapson and Cruickshank 1947) or higher concentration of NH_4^+ triggers the reversible production of GDP-mannose which is a key intermediate enzyme required for vitamin C synthesis (Bonin et al. 1997; Conklin et al. 1999b). Our results corroborate the findings of previous studies who reported lower vitamins C in lettuce and Kacip Fatimah (*Labisia pumila* Benth) when cultivated in organic nutrient source (Ibrahim et al. 2013).

Other vitamins such as riboflavin, pantothenic acid and folates are also important vitamins for the normal functioning of human body. Watson and Noggle. (1947) investigated the effect of different mineral nutrient solutionms on riboflavin in oat, consisting of Hoagland solution as a control and remaining were deficient in a particular mineral such as K, Ca, Mg, NO_3^-, SO_4^{2-} and PO_4^3. They observed that mineral deficiencies affected riboflavin synthesis and found a positive relationship between N uptake and riboflavin synthesis in oats leaves. In present study, we observed lower riboflavin contents and higher pantothenic acid and folates in DD grown lettuce. Lower riboflavin contents in present study might be due to NO_3^--N deficiency or lower NO_3^-/NH_4 ratio in DD treatment which reduced riboflavin contents in lettuce. Additionally, total N-uptake in lettuce grown in DD was significantly lower than NS treatment, which support the arguments of Watson and Noggle (1947).

Similarly, lettuce varieties exhibited variation in folate contents and other vitamins (Kim et al. 2016) and were consistent with study conducted by Mou (2009) who reported that Romaine leaf contains higher riboflavin, pantothenic acid and folates content than a crisphead variety. He reported that these vitamins concentration ranges from 0.03 mg - 0.08 mg / 100 g sample for riboflavin, 0.09 - 0.15 mg / 100 g sample for pantothenic acid and 29 - 136 µg / 100 g sample for folates. In present study, riboflavin, and pantothenic acid were lower (0.002-0.005 mg g^{-1} sample, 0.015-0.033 mg g^{-1} sample) and folate contents were much higher (197.62-510.91 µg g^{-1} sample) as observed by Mou (2009).

Ibrahim et al. (2013) investigated the effects of chicken dung (10: 10: 10 NPK) and inorganic fertilizers (15: 15: 15 NPK) on total phenolics and total antioxidants in Kacip Fatimah (*Labisia pumila* Benth) and observed higher concentration of total phenolics and antioxidants in chicken dung grown crop than crop grown on inorganic fertilizers. We also observed the similar trend of higher total phenolics and total antioxidants in DD grown lettuce than NS which might be due to slow or limited N availability, which might have changed metabolism towards carbon-containing compounds and non-N-containing secondary metabolites such as phenolics and terpenoids (Haukioja et al. 1998). Results of present study are consistent with studies reported by Ibrahim et al. (2013) and Haukioja et al. (1998) who reported higher phenolic contents in organically produced crop. Liu et al. (2007) examined twelve green and red lettuce cultivars for their phenolic contents and reported higher total phenolic contents in red type than green type of lettuce which varied from 21-74 mg g^{-1} sample FW. Similarly, Mampholo et al. (2016) also found significant variation in total antioxidants (15 -127 mg TEAC (Trolox equivalent antioxidant capacity) 100 g^{-1} FW) production among the lettuce varieties In present study, total phenolic and total antioxidants contents in lettuce varieties varied from 1.4 - 3 mg g^{-1} sample FW and 22 - 34 mg g^{-1} sample FW respectively, which were lower than reported by Liu et al. (2007) and Mampholo et al. (2016).

Phenolic acid such as chicoric acid has immune-stimulatory properties, promote phagocytosis activity (Bauer et al. 1989b) and also helpful to inhibit the HIV replication (Healy et al. 2009b), whereas, chlorogenic acid is helpful against hepatitis B virus in human (Zuo et al. 2015). Quercetin and their derivatives, found in fruits and vegetables, have unique biological properties that may not only improve mental and physical health but also diminish infection risk (Davis et al. 2009).

Several studies testified that polyphenols have anti-inflammatory and therapeutic properties that may reduce cardiovascular disease, neurodegenerative disorders, cancer, and obesity (Pérez-Jiménez et al. 2010). Biosynthesis of polyphenolic compounds is highly dependent on N fertilization practices and a lower availability of N is associated with increased phenolic compounds. For instance, in a previous study conducted by Mollavali et al. (2018) reported augmented concentration of various polyphenols such as quercetin-3,4′-di-O-β-D-glucoside and quercetin-4′-O-β-D-glucoside in onion plants. This increased polyphenol concentration is closely associated with increased phenylalanine ammonia lyase (PAL) enzyme activity in the plants grown in predominantly higher NH_4^+-N solution. Higher NH_4^+-N might have stimulated biosynthesis of phenolic compounds and increased expression of soluble peroxidase and superoxide dismutase enzyme under oxidative stress condition (Mihaljević et al. 2011). In present study, higher NH_4^+-N stress in DD solution might have triggered a stress response and stimulated PAL enzyme that enhanced biosynthesis of polyphenols and total antioxidants. Similarly, Durazzo et al. (2014) also observed higher concentration of polyphenols such as Lutein and Caffeic acid and lower chlorogenic acid and Quercetin in organically grown lettuce. Likewise, Rajashekar et al. (2012) also reported higher concentration of polyphenols concentration in organically grown lettuce as compared to conventionally grown lettuce. They also observed higher concentration of polyphenols such as chicoric acid (0.2 - 1.1 mg g^{-1} FW), chlorogenic acid (0.15 – 0.5 mg g^{-1} FW) and quercetin 3-O-glucoside (0.03-0.10 mg g^{-1} FW) in organically grown lettuce, whereas, Luteolin-7-O-glucoside (0.01 – 0.02 mg g^{-1} FW) was higher in conventionally grown lettuce. In present study, we also observed significant variation in Chicoric acid, Chlorogenic acid, Luteolin 3-O-glucoside and Quercetin 3-O-(malonyl)-b-D-glucoside concentration in the lettuce grown in DD than inorganically fertilized lettuce.

4.3. Conclusion and recommendations

Adequate and balanced nutrients availability are important for growth, development and yield of crops especially when grown in hydroponic systems. Lettuce plants grown in NS showed higher LA chlorophyll content, root dry weight, yield, minerals and vitamin C compared to organic nutrient solution such as DD. However, when DD was used as a sole mineral nutrient source for lettuce production, it resulted in lower LA, chlorophyll content, root dry weight, final yield, mineral and vitamin C contents. These reduced plant growth parameters and minerals contents could be associated with high NH_4^+: NO_3^- in DD treatment solution which showed toxic effects on root growth, increased pH reduced micronutrients uptake and consequently resulted in reduced final lettuce yield and mineral concentration. On the other hand, plants in the DD treatment showed superior performance as a sole nutrient source and exhibited higher vitamins (riboflavin, pantothenic acid folates), phenolics, flavonoids (chicoric acid, Chlorogenic acid, Quercetin-3-β-d-gluconide, luteolin, quercetin-3-glucoside and quercetin-β-malonyl), total antioxidants and lower NO_3^- contents. This research demonstrates that DD could be used as sustainable nutrient source to improve phytochemical profile of lettuce in hydroponics which can improve human health. Further research is required to reduce NH_4^+: NO_3^- in DD without dilution to establish the utilization of DD as a sole source of mineral nutrients in hydroponics, because the dilution can reduce the other macro and micronutrients in DD which results in poor plant performance.

4.4. References

Adams P (2002) Nutritional control in hydroponics. In: Savvas, D., Passam, H.C. (Eds.), Hydroponic Production of Vegetables and Ornamentals. Embryo Publications, Athens, Greece,

Ahmad R, Jabeen N (2009) Demonstration of growth improvement in sunflower (helianthus annuus l.) By the use of organic fertilizers under saline conditions

Ahring B (2003) Preface. In: Ahring BK (ed) Biomethanation II. Springer, Berlin.

Akhavan HR, Barzegar M (2017) Determination of water-soluble vitamins in 15 Iranian pomegranate cultivars and their variation after pasteurization and cold storage. . Int Food Res J 24:1429

Alburquerque JA, de la Fuente C, Ferrer-Costa A, et al (2012) Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass and bioenergy,. Elsevier 40:181–189

Arnon DI (1949) Copper enzymes in isolated chloropolasts. polyphenoloxidase in (Beta vulgaris). Plant Physiol 1–15

Bachmann S, Uptmoor R, Eichler-Löbermann B (2016) Phosphorus distribution and availability in untreated and mechanically separated biogas digestates. Sci Agric 73:9–17

Bachmann S, Wentzel S, Eichler-Löbermann B (2011) Codigested dairy slurry as a phosphorus and nitrogen source for Zea mays L. and Amaranthus cruentus L. J Plant Nutr Soil Sci 174:908–915

Barker A V., Eaton TE, Meagy MJ, et al (2017) Variation of mineral nutrient contents of modern and heirloom cultivars of cabbage in different regimes of soil fertility. J Plant Nutr 40:2432–2439

Barker A V., Maynard DN, Lachman WH (1967) Induction of tomato stem and leaf lesions, and potassium deficiency, by excessive ammonium nutrition. Soil Sci 103:319–327

Barth C, Gouzd ZA, Steele HP, Imperio RM (2010) A mutation in GDP-mannose pyrophosphorylase causes conditional hypersensitivity to ammonium, resulting in Arabidopsis root growth inhibition, altered ammonium metabolism, and hormone homeost. J Exp Bot 61:379-394.

Barzee TJ, Edalati A, El-Mashad H, et al (2019) Digestate biofertilizers support similar or higher tomato yields and quality than mineral fertilizer in a subsurface drip fertigation system. Front Sustain Food Syst 3:58

Bauer A, Mayr H, Hopfner-Sixt K, Amon T (2009) Detailed monitoring of two biogas plants and mechanical solid–liquid separation of fermentation residues. J Biotechnol 142:56–63

Bauer R, Reminger P, Jurcic K, Wagner H (1989a) Influence of Echinacea extracts on phagocytic activity. Z Phytother 10:43–48

Bauer R, Reminger P, Jurcic K, Wagner H (1989b) Influence of Echinacea extracts on phagocytic activity. Z Phytother 10:43–48

Benbrook C, Zhao X, Yáñez J, et al (2008) New Evidence Confirms the Nutritional Superiority of Plant-Based Organic Foods. State of Science Review; The Organic Center: Boulder, CO, USA,

Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70–76

Bittsánszky A, Pilinszky K, Gyulai G, Komives T (2015) Overcoming ammonium toxicity. Plant Sci 231:184–190

Bloch D, Monshausen G, Singer M, et al (2011) Nitrogen source interacts with ROP signalling in

root hair tip-growth. Plant, Cell Environ 34:76–88

Bonin CP, Potter I, Vanzin GF, Reiter W-D (1997) The MUR1 gene of Arabidopsis thaliana encodes an isoform of GDP-D-mannose-4, 6-dehydratase, catalyzing the first step in the de novo synthesis of GDP-L-fucose. Proceedings of the National Ac. Plant Biol 94:2085–2090

Boonpangrak S, Lalitmanat S, Suwanwong Y, et al (2016) Analysis of Ascorbic Acid and Isoascorbic Acid in Orange and Guava Fruit Juices Distributed in Thailand by LC-IT-MS/MS. Food Anal Methods 9:1616–1626

Börjesson P, Berglund M (2007) Environmental systems analysis of biogas systems—Part II: The environmental impact of replacing various reference systems. Biomass and Bioenergy 31:326–344

Bottoms TG, Smith RF, Cahn MD, Hartz TK (2012) Nitrogen requirements and N status determination of lettuce. HortScience 12:1768–1774

British Nutrition F (2009) Minerals and trace elements. Assessed on: March 06, 2020. Available at: https://www.nutrition.org.uk/nutritionscience/nutrients-food-and-ingredients/minerals-and-trace-elements.html?showall=1&limitstart

Britto DT, Kronzucker HJ (2002) NH4+ toxicity in higher plants: a critical review. J Plant Physiol 159:567–584

Bryson GM, Mills HA, Sasseville DN, et al (2014) Plant analysis handbook III. Athens, GA: Micro-Macro Publishing

Buchanan D, Omaye S (2013) Comparative Study of Ascorbic Acid and Tocopherol Concentrations in Hydroponic- and Soil-Grown Lettuces. Food Nutr Sci 10:1047–1053.

Cano A, Acosta M, Arnao MB (2003) Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (Lycopersicon esculentum Mill.). Postharvest Biol

Technol 28:59–65

Carr AC, Vissers MCM (2013) Synthetic or food-derived vitamin C—are they equally bioavailable? Nutrients 5:4284–4304

Chantigny M, Angers D, Belanger G, et al (2008) Yield and nutrient export of grain corn fertilized with raw and treated liquid swine manure. Agron J 1303–1309. https://doi.org/10.2134/agronj2007.0361

Chantigny MH, Angers DA, Rochette P, et al (2007) Gaseous Nitrogen Emissions and Forage Nitrogen Uptake on Soils Fertilized with Raw and Treated Swine Manure. J Environ Qual 36:1864–1872

Chapman HD, Pratt PF (1962) Methods of analysis for soils, plants and waters. Soil Sci 93:68

Cometti NN, Bremenkamp DM, Galon K, et al (2013) Cooling and concentration of nutrient solution in hydroponic lettuce crop. Hortic Bras 31:287–292

Conklin PL, Norris SR, Wheeler GL, et al (1999a) Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis. Proc Natl Acad Sci 96:4198–4203

Conklin PL, Norris SR, Wheeler GL, et al (1999b) Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis

Coronel G, Chang M, Rodríguez-Delfín A (2008) Nitrate reductase activity and chlorophyll content in lettuce plants grown hydroponically and organically. In International Symposium on Soilless Culture and Hydroponics 843. pp 137–144

Correia ECS (2013) Reação de cultivares de alface do grupo americano a Meloidoygine incognita, M. javanica e M. enterolobii (55f. Dissertação (Mestrado em Agronomia), Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, Brazil).

Craig W, Beck L (1999) Phytochemicals: Health Protective Effects. Can J Diet Pract Res 60:78–

84

Cuevas-Rodri´guez EO, Rodri´guez R, Dia VP, et al (2010) Inhibition of Pro-inflammatory Responses and Antioxidant Capacity of Mexican Blackberry (Rubus spp.) Extracts. J Agric Food Chem 58:9542–9548. https://doi.org/10.1021/jf102590p

Dai J, Mumper RJ (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15:7313–7352

Davis JM, Murphy EA, Carmichael MD (2009) Effects of the dietary flavonoid quercetin upon performance and health. Curr Sport Med Rep 8:206–213

Dias JS, Ryder E (2011) World Vegetable Industry: Production, Breeding, Trends. Hort Rev 38:299–356

Drennan MF, DiStefano TD (2010) Characterization of the curing process from high-solids anaerobic digestion. Bioresour Technol 2:537–544

Duarte TL, Lunec J (2005) When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C. Free Radic Res 39:671–686

Durazzo A, Azzini E, Lazzé MC, et al (2014) Antioxidants in Italian head lettuce (lactuca sativa var. capitatal.) Grown in organic and conventional systems under greenhouse conditions. J Food Biochem 38:56–61

El-Shinawy MZ, Gawish SM (2006) Effect of commercial organic nutrient solutions on growth and chemical composition of lettuce under agricultural soilless system. Egypt J Hortic 33:19

Environmental Protection Agency (1979) Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020

Errebhi M, Wilcox GE (1990) Plant species response to ammonium-nitrate concentration ratios. J Plant Nutr 13:1017–1029

Esteban R, Ariz I, Cruz C, Moran J (2016) Review: Mechanisms of ammonium toxicity and the quest for tolerance. Plant Sci 248:92–101.

Fan J, Xiao J, Liu D, et al (2017) Effect of application of dairy manure, effluent and inorganic fertilizer on nitrogen leaching in clayey fluvo-aquic soil: A lysimeter study. Sci. Total Env 592:206–214

FAO (2018) The state of food insecurity in the world 2018. Available at: http://www.fao.org/state-of-food-security-nutrition. Assessed on: (April 20, 2020). Public Library of Science

Fernández-Cirelli A, Arumí JL, Rivera D, Boochs PW (2009) Environmental effects of irrigation in arid and semi-arid regions. Chil J Agric Res 27–40

Fishman M, Friedman L (1989) Methods for determination of inorganic substances in water and fluvial sediments. US Department of the Interior, Geological Survey.

Forge T, Kenney E, Hashimoto N, et al (2016) Compost and poultry manure as preplant soil amendments for red raspberry: Comparative effects on root lesion nematodes, soil quality and risk of nitrate leaching. Agric Ecosyst Environ 223:48–58

Fullbloom (2020) Hydroponic Systems 101 | Learn The Basics of Hydroponics. Available at: https://www.fullbloomgreenhouse.com/hydroponic-systems-101/ (Assessed on July 05, 2020).

Furukawa Y, Hasegawa H (2006) Response of Spinach and Komatsuna to Biogas Effluent Made from Source-Separated Kitchen Garbage. J Environ Qual 35:1939–1947

Gagliardini E, Buelli S, Benigni A (2011) Endothelin in chronic proteinuric kidney disease. Contrib Nephrol 172:171-184.

Garbin ML, Dillenburg LR (2008) Effects of different nitrogen sources on growth, chlorophyll

concentration, nitrate reductase activity and carbon and nitrogen distribution in Araucaria angustifolia. Brazilian J Plant Physiol 20:295–303

Gavrilova V, Kajd M, Gjamovski V, Stefova M (2011) Separation, Characterization and Quantification of Phenolic Compounds in Blueberries and Red and Black Currants by HPLCÀDADÀESI-MS n. J Agric Food Chem 59:4009–4018

Gell K, van Groenigen J, Cayuela ML (2011) Residues of bioenergy production chains as soil amendments: immediate and temporal phytotoxicity. J Hazard Mater 186:2017–2025

Georgacakis D, Sievers DM, Iannotti EL (1982) Buffer stability in manure digesters. Agric Wastes 4:427–441

Gerendás J, Zhu Z, Bendixen R, et al (1997) Physiological and biochemical processes related to ammonium toxicity in higher plants. J Plant Nutr Soil Sci 160:239–251

Gliszczyńska-Świgło A (2007) Folates as antioxidants. Food Chem 101:1480–1483

Goddek S, Schmautz Z, Scott B, et al (2016) The effect of anaerobic and aerobic fish sludge supernatant on hydroponic lettuce. Agronomy 2:37

Gómez X, Cuetos MJ, García AI, Morán A (2007) An evaluation of stability by thermogravimetric analysis of digestate obtained from different biowastes. J Hazard Mater 149:97–105

Gong W, Yan X, Wang J, et al (2011) Long-term applications of chemical and organic fertilizers on plant-available nitrogen pools and nitrogen management index. Biol Fertil Soils 47:767–775

Gupta UC, Gupta SC (2014) Sources and deficiency diseases of mineral nutrients in human health and nutrition: a review. Pedosphere 24:13–38

Hao XJ, Hong JP, Gao WJ (2007) Effect of biogas slurry and biogas residues on quality of

greenhouse mini cucumber. [J] Soil Fertil Sci China 5:9

Hariadi YC, Nurhayati AY, Hariyani P (2016) Biophysical monitoring on the effect on different composition of goat and cow manure on the growth response of maize to support sustainability. Agric Agric Sci Procedia 118–127

Harris E (2001) Copper homeostasis: the role of cellular transporters. Nutr Rev 59:281–285

Hassan SA, Mijin S, Yusoff UK, et al (2012) Nitrate, ascorbic acid, mineral and antioxidant activities of Cosmos caudatus in response to organic and mineral-based fertilizer rates. Molecules 17:7843–7853

Haukioja E, Ossipov V, Koricheva J, et al (1998) Biosynthetic origin of carbon-based secondary compounds: cause of variable responses of woody plants to fertilization? Chemoecology 8:133–139

Healy EF, Sanders J, King PJ, Edwards Robinson WJ (2009a) A docking study of L-chicoric acid with HIV-1 integrase. J Mol Graph Model 27:584–589

Healy EF, Sanders J, King PJ, Robinson Jr WE (2009b) A docking study of L-chicoric acid with HIV-1 integrase. J Mol Graph Model 27:584–589

Heimler D, Isolani L, Vignolini P, Romani A (2009) Polyphenol content and antiradical activity of Cichorium intybus L. from biodynamic and conventional farming. Food Chem 114:765–770

Herrera E, Jiménez R, Aruoma OI, et al (2009) Aspects of Antioxidant Foods and Supplements in Health and Disease. Nutr Rev 67:140–144

Hjorth M, Christensen K V., Christensen ML, Sommer SG (2010) Solid—liquid separation of animal slurry in theory and practice. A review. Agron Sustain Dev 30:153–180

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil.

Circular. California agricultural experiment station, 347(2nd edit).

Hobson P, Wheatley A (1992) Anaerobic digestion – modern theory and practice. Elsevier Appl Sci 269

Hokkaido (2003) Research report of environment, resources, and recycling in Hokkaido, Sapporo. Civil Engineering Institute of Hokkaido, Sapporo (in Japanese).

Hoque MM, Ajwa HA, Smith R (2007) Nitrite and ammonium toxicity on lettuce grown under hydroponics. Commun Soil Sci Plant Anal 39:207–216

Hord NG, Tang Y, Bryan NS (2009a) Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 90:1-10.

Hord NG, Tang Y, Bryan NS (2009b) Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 90:1–10

Hung HC, Joshipura KJ, Jiang R, et al (2004) Fruit and vegetable intake and risk of major chronic disease. J Natl Cancer Inst 96:1577–1584

Ibrahim MH, Jaafar HZ, Karimi E, Ghasemzadeh A (2013) Impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kacip Fatimah (Labisia pumila Benth). Molecules 18:10973–10988

Insam H, Gómez-Brandón M, Ascher J (2015) Manure-based biogas fermentation residues– Friend or foe of soil fertility? Soil Biol Biochem 84:1–14

IPL (2020) Hydroponics Advantages And Disadvantages. Available at: https://www.ipl.org/essay/Hydroponics-Advantages-And-Disadvantages-PKNBD2NFCEDR (Assessed on July 05, 2020).

Joshi R, Adhikari S, Patro BS, et al (2001) Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. Free Radic Biol Med 30:1390–1399

Kapoulas N, Koukounaras A, Ilić ZS (2017) Nutritional quality of lettuce and onion as companion plants from organic and conventional production in north Greece. ElsevierScientia Hortic 219:310–318

Karakaya S (2004) Bioavailability of Phenolic Compounds. Crit Rev Food Sci Nutr 44:453–464

Kempinski CF, Haffar R, Barth C (2011) Toward the mechanism of NH4+ sensitivity mediated by Arabidopsis GDP-mannose pyrophosphorylase. Plant, Cell Environ 34:847–858

Kim MJ, Moon Y, Tou JC, et al (2016) Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.). J Food Compos Anal 49:19–34

Kirchmann H, Bernal M (1997) Organic waste treatment and C stabilization efficiency. Soil Biol Biochem 29:1747–1753

Klouberta V, Rink. L (2015) Zinc as a micronutrient and its preventive role of oxidative damage in cells. Food Funct 6:3195–3204

Knapp Haraldsen T, Andersen U, Krogstad T, Sørheim R (2011) Liquid digestate from anaerobic treatment of source-separated household waste as fertilizer to barley. Waste Manag Res 29:1271–1276

Koester RP, Skoneczka JA, Cary TR, et al (2014) Historical gains in soybean (Glycine max Merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. J Exp Bot 12:3311–3321

Kris-Etherton PM, Hecker KD, Bonanome A, et al (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 9:71–88

Krupa S V. (2003) Effects of atmospheric ammonia (NH3) on terrestrial vegetation: a review. Envionmental Pollut 124:179–221

Lafleur B, Thiffault E, Paré D, et al (2012) Effects of hog manure application on the nutrition

and growth of hybrid poplar (Populus spp.) and on soil solution chemistry in short-rotation woody crops. Agric Ecosyst environmen 155:95–104

Lam V, Watanabe T, Phan T, Khai L (2002) A case study: introduction of low-cost biogas digester to small-scale farming systems

Lampkin N (1990) Organic farming. Farming press books. Ipswich. United Kingdom.

Li B, Li G, Kronzucker HJ, et al (2014) Ammonium stress in Arabidopsis: signaling, genetic loci, and physiological targets. Trends Plant Sci 19:107–114

Li B, Li Q, Xiong L, et al (2012) Arabidopsis plastid AMOS1/EGY1 integrates abscisic acid signaling to regulate global gene expression response to ammonium stress. Plant Physiol 160:2040–2051

Li BH, Shi. WM (2007) Effects of elevated NH4+ on Arabidopsis seedlings different in accessions. Acta Pedofil Sin 44:508–515

Li Q, Li X, Tang B, Gu M (2018) Growth responses and root characteristics of lettuce grown in aeroponics, hydroponics, and substrate culture. Horticulturae 4:35

Liedl BE, Cummins M, Young A, et al (2004) Hydroponic lettuce production using liquid effluent from poultry waste bioremediation as a nutrient source. In VII International Symposium on Protected Cultivation in Mild Winter Climates: Production, Pest Management and Global Competition 659. pp 721–728

Liedl BE, Wilfong K, Taylor C, Mazzaferro K (2006) Liquid effluent from poultry waste bioremediation as a nutrient source for hydroponic cucumber production. HortScience 4:997A – 997

Lin D, Xiao M, Zhao J, et al (2016) An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules 21:1374

Lind OP, Hultberg M, Bergstrand KJ, et al (2020) Biogas Digestate in Vegetable Hydroponic Production: pH Dynamics and pH Management by Controlled Nitrification. Waste and Biomass Valorization 1–11

Liu WK, Yang QC, Du L (2009) Soilless cultivation for high-quality vegetables with biogas manure in China: Feasibility and benefit analysis. Renewable Agriculture and Food Systems,. Renew Agric Food Syst 300–307

Liu WK, Yang QC, du Lian F, et al (2011) Nutrient supplementation increased growth and nitrate concentration of lettuce cultivated hydroponically with biogas slurry. Acta Agric Scand Sect B Soil Plant Sci 61:391–394

Liu X, Ardo S, Bunning M, et al (2007) Total phenolic content and DPPH radical scavenging activity of lettuce (Lactuca sativa L.) grown in Colorado. LWT Food Sci Technol 40:552–557

Liu Y, Lai N, Gao K, et al (2013) Ammonium inhibits primary root growth by reducing the length of meristem and elongation zone and decreasing elemental expansion rate in the root apex in Arabidopsis thaliana. PLoS One 8:e61031.

Liu YJ, Tong YP, Zhu YG, et al (2006) Leaf chlorophyll readings as an indicator for spinach yield and nutritional quality with different nitrogen fertilizer applications. J Plant Nutr 29:1207–1217

Llorach R, Martínez-Sánchez A, Tomás-Barberán FA, et al (2008) Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem 108:1028–1038

Loqué D, Mora SI, Andrade SL, et al (2009) Pore mutations in ammonium transporter AMT1 with increased electrogenic ammonium transport activity. J Biol Chem 284:24988–24995

Loria ER, Sawyer JE, Barker DW, et al (2007) Use of anaerobically digested swine manure as a nitrogen source in corn production. In: Agronomy Journal. pp 1119–1129

Madeira AC, De Varennes A (2005) Use of chlorophyll meter to assess the effect of nitrogen on sweet pepper development and growth. J Plant Nutr 28:1133–1144

Makádi M, Orosz A, Tomócsik V (2012) Digestate: A New Nutrient Source - Review, Biogas, Dr. Sunil Kumar (Ed.), ISBN: 978-953-51-0204-5, InTech, Available from: http://www.intechopen.com/books/biogas/digestate-a-new-nutrient-source-review

Makádi M, Tomócsik A, Kátai J, et al (2008) Nutrient cycling by using residues of bioenergy production - effects of biogas-digestate on plant and soil parameters. Cereal Res Commun 1807–1810

Mampholo BM, Maboko MM, Soundy P, Sivakumar D (2016) Phytochemicals and Overall Quality of Leafy Lettuce (Lactuca sativa L.) Varieties Grown in Closed Hydroponic System. J Food Qual 39:805–815

Man J, Shi Y, Yu Z, Zhang Y (2015) Dry matter production, photosynthesis of flag leaves and water use in winter wheat are affected by supplemental irrigation in the Huang-Huai-Hai Plain of China. PLoS One 10:e0137274.

Mapson LW, Cruickshank EM (1947) Effect of various salts on the synthesis of ascorbic acid and carotene in cress seedlings. Biochem J 41:197-205.

Masarirambi MT, Hlawe MM, Oseni OT, Sibiya TE (2010) Effects of organic fertilizers on growth, yield, quality and sensory evaluation of red lettuce (Lactuca sativa L.) ‘Veneza Roxa.’ Agric Biol J N Am, 1:1319–1324

Massé DI, Talbot G, Gilbert Y (2011) On farm biogas production: A method to reduce GHG emissions and develop more sustainable livestock operations. Anim Feed Sci Technol 436–

445

Mayer-Foulkes DA (2011) A survey of macro damages from non-communicable chronic diseases: Another challenge for global governance. Glob Econ J 11:1–26

Meagy MJ, Eaton TE, Barker A V. (2016) Assessment of mineral nutrient concentrations of lettuce in response to cultivar selection and fertilization in greenhouse production. J Plant Nutr 39:1796–1808

Medicine I of (2005) Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Institute of Medicine, Washington D.C. National Academies Press (US).

Medicine I of (1997) Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Institute of Medicine, Washington, D.C. National Academies Press (US).

Medicine I of (1998) Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Institute of Medicine, Washington, D.C. National Academies Press (US).

Medina E (1984) Nutrient balance and physiological processes at the leaf level. In Physiological ecology of plants of the wet tropics (pp. 139-154). Springer, Dordrecht. pp 139–154

Menardo S, Gioelli F, Balsari P (2011) The methane yield of digestate: effect of organic loading rate, hydraulic retention time, and plant feeding. Bioresour Technol 102:2348–2351

Mihaljević S, Radić S, Bauer N, et al (2011) Ammonium-related metabolic changes affect somatic embryogenesis in pumpkin (Cucurbita pepo L.). J Plant Physiol 168:1943–1951

Miller J, Rucker R (2012) Pantothenic acid. In: Erdman JW, Macdonald IA, Zeisel SH, eds. Present Knowledge in Nutrition. 10th ed. Washington, DC: Wiley-Blackwell:375-90.

Mohankumar JB, Uthira L, Maheswari SU (2018) Total phenolic content of organic and conventional green leafy vegetables. J Nutr Hum Heal 2:1–6

Mollavali M, Perner H, Rohn S, et al (2018) Nitrogen form and mycorrhizal inoculation amount and timing affect flavonol biosynthesis in onion (Allium cepa L.). Mycorrhiza 28:59–70

Möller K, Schulz R, Müller T (2010) Substrate inputs, nutrient flows and nitrogen loss of two centralized biogas plants in southern Germany. Nutr Cycl Agroecosystems 87:307–325

Möller K, Stinner W, Deuker A, Leithold G (2008a) Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. Nutr Cycl Agroecosystems 82:209–232

Möller K, Stinner W, Deuker A, Leithold G (2008b) Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. Nutr Cycl Agroecosystems 82:209–232

Morris MC, Evans DA, Tangney CC, et al (2006) Associations of vegetable and fruit consumption with age-related cognitive change. Neurology 67:1370–1376

Mou B (2009) Nutrient content of lettuce and its improvement. Curr Nutr Food Sci 5:242-248.

Mou B, Ryder EJ (2004) Relationship between the nutritional value and the head structure of lettuce. Acta Hortic 637:361–7

Mulla D, Birr A, Randall G, et al (2001) Impacts of animal agriculture on water quality: technical work paper. Minnesota Environmental Quality Board, Minnesota Planning (Agency), St. Paul.

Nicola S, Hoeberechts J, Fontana E (2005) Comparison between traditional and soilless culture systems to produce rocket (Eruca sativa) with low nitrate content. In International Symposium on Soilless Culture and Hydroponics; International Society for. pp 549–555.

Nicoletto C, Santagata S, Zanin G, Sambo P (2014) Effect of the anaerobic digestion residues use on lettuce yield and quality. Sci Hortic (Amsterdam) 180:207–213

Nicolle C, Carnat A, Fraisse D, et al (2004) Characterisation and variation of antioxidant micronutrients in lettuce (Lactuca sativa folium). J Sci Food Agric 84:2061–2069

Nkoa R (2014) Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. Agron Sustain Dev 34:473-492.

Norenberg MD, Rama Rao K V., Jayakumar AR (2009) Signaling factors in the mechanism of ammonia neurotoxicity. Metab. Brain Dis. 24:103–117

Olsen KM, Lea US, Slimestad R, et al (2008) Differential expression of four Arabidopsis PAL genes; PAL1 and PAL2 have functional specialization in abiotic environmental-triggered flavonoid synthesis. J Plant Physiol 165:1491–1499

Paavola T, Rintala J (2008) Effects of storage on characteristics and hygienic quality of digestates from four co-digestion concepts of manure and biowaste. Bioresour Technol 99:7041–7050

Pan Z, Qi G, Andriamanohiarisoamanana FJ, et al (2018) Potential of anaerobic digestate of dairy manure in suppressing soil-borne plant disease. Anim Sci J 89:1512–1518

Pavia M, Pileggi C, Nobile CGA, Angelillo IF (2006) Association between fruit and vegetable consumption and oral cancer: a meta-analysis of observational studies. Am J Clin Nutr 83:1126–1134

Pellati F, Benvenuti S, Magro L, et al (2004) Analysis of phenolic compounds and radical scavenging activity of Echinacea spp. J Pharm Biomed Anal 35:289–301

Pérez-Jiménez J, Neveu V, Vos F, Scalbert A (2010) Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. Eur J Clin Nutr 64:112–120

Petropoulos SA, Chatzieustratiou E, Constantopoulou E, Kapotis G (2016) Yield and quality of

lettuce and rocket grown in floating culture system. Not Bot Horti Agrobot Cluj-Napoca 44:603–612

Pognani M, D'Imporzano G, Scaglia B, Adani F (2009) Substituting energy crops with organic fraction of municipal solid waste for biogas production at farm level: A full-scale plant study. Process Biochem 44:817–821

Przygocka-Cyna K, Grzebisz W, Lukowiak R, Biber M (2018) Biomass ash and biogas digestate bio-fertilizers as a source of nutrients for light acid soil–an exhaustion test. J Elem 23:413–428

Qi X, Zhang S, Wang Y, Wang R (2005) Advantages of the integrated pig-biogas-vegetable greenhouse system in North China. Ecol Eng 24:175–183

Qin C, Qian W, Wang W, et al (2008) GDP-mannose pyrophosphorylase is a genetic determinant of ammonium sensitivity in Arabidopsis thaliana

Qu H, Madl RL, Takemoto DJ, et al (2005) Lignans are involved in the antitumor activity of wheat bran in colon cancer SW480 cells. J Nutr 3:598–602

Rajashekar CB, Oh M-M, Carey EE (2012) Organic Crop Management Enhances Chicoric Acid Content in Lettuce. Food Nutr Sci 3:1296–1302

Rezk BM, Haenen GR, van der Vijgh WJ, Bast A (2003) Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophores. FEBS Letters. Elsevier 555:601– 605

Roberts S (2018) What Is the Main Function of Phosphorus in the Body? Assessed on: March 06, 2020. Available at: https://healthyeating.sfgate.com/main-function-phosphorus-body-5789.html

Ronga D, Setti L, Salvarani C, et al (2019) Effects of solid and liquid digestate for hydroponic

baby leaf lettuce (Lactuca sativa L.) cultivation. Sci Hortic (Amsterdam) 172–181

Roosta HR, Schjoerring JK (2007) Effects of ammonium toxicity on nitrogen metabolism and elemental profile of cucumber plants. J Plant Nutr 30:1933–1951

Sacks FM, Willett WC, Smith A, et al (1998) Effect on Blood Pressure of Potassium, Calcium, and Magnesium in Women With Low Habitual Intake. Hypertension 31:131–138

Salminen E, Rintala J, Härkönen J, et al (2001) Anaerobically digested poultry slaughterhouse wastes as fertiliser in agriculture. Bioresour Technol 78:81–88

Santamaria P (2006) Nitrate in vegetables: Toxicity, content, intake and EC regulation. J. Sci. Food Agric. 86:10–17

Santos FT, Goufo P, Santos C, et al (2016) Comparison of five agro-industrial waste-based composts as growing media for lettuce: Effect on yield, phenolic compounds and vitamin C. Food Chem 209:293–301

Sapkota S, Sapkota S, Liu Z (2019) Effects of nutrient composition and lettuce cultivar on crop production in hydroponic culture. Horticulturae 5:72

Savvas D, Passam HC, Olympios C, et al (2006) Effects of Ammonium Nitrogen on Lettuce Grown on Pumice in a Closed Hydroponic System

Scaglia B, Pognani M, Adani F (2017) The anaerobic digestion process capability to produce biostimulant: the case study of the dissolved organic matter (DOM) vs. auxin-like property. Sci Total Environ 589:36-45.

Scott J, Rébeillé F, Fletcher J (2000) Folic acid and folates: The feasibility for nutritional enhancement in plant foods. J. Sci. Food Agric. 80:795–824

Seefeldt S (2015) Crop Production and Soil Mangement Series Steven Seefeldt, Extension Faculty, Agriculture and Horticulture. Assessed on: September 14, 2020. Available at:

http://cespubs.uaf.edu/index.php/download_file/1025/.

Shohag MJI, Wei Y, Yang X (2012) Changes of Folate and Other Potential Health-Promoting Phytochemicals in Legume Seeds As Affected by Germination. J Agric Food Chem 60:9137–9143

Silber A, Bar-Tal A (2008) Nutrition of substrate-grown plants. Soil Cult Theory Pract Elsevier, San Diego, CA, 4:291-339.

Slamet W, Purbajanti ED, Darmawati A, Fuskhah E (2017) Leaf area index, chlorophyll, photosynthesis rate of lettuce (Lactuca sativa L) under N-organic fertilizer. Indian J Agric Res 4:365–369

Soetan KO, Olaiya CO, Oyewole OE (2010) The importance of mineral elements for humans, domestic animals and plants: A review. African J food Sci 5:200-222.

Somasundaram E, Amanullah MM, Vaiyapuri K, et al (2007) Influence of organic sources of nutrients on the yield and economics of crops under maize based cropping system. J Appl Sci Res 1774-1777.

Sommer SG, Husted S (1995a) A simple model of pH in slurry. J Agric Sci 124:447–453

Sommer SG, Husted S (1995b) The chemical buffer system in raw and digested animal slurry

Southon S (2000) “Increased Fruit and Vegetable Consumption within the EU: Potential Health Benefits,” Food Research International. 33:211-217.

Squire GR, Ong CK, Monteith JL (1987) Crop growth in semi-arid environment. In: Proceedings of 7th International Workshop, 7-11 April, 1986, International Crops Research Institute for Semi-Arid Tropics. Patancheru, Hyderabad, pp 219-231.

Stinner W, Möller K, Leithold G (2008) Effects of biogas digestion of clover/grass-leys, cover crops and crop residues on nitrogen cycle and crop yield in organic stockless farming

systems. Eur J Agron 29:125–134

Stoknes K, Scholwin F, Krzesiński W, et al (2016) Efficiency of a novel "Food to waste to food" system including anaerobic digestion of food waste and cultivation of vegetables on digestate in a bubble-insulated greenhouse. Waste Manag 466–476

Su YY, Lu Y, Shi WS (2008) Effect of biogas fertilizer on yield and quality of lettuce in soilless culture. [J] Soil Fertil Sci China 1

Sulaiman SF, Yusoff NAM, Eldeen IM, et al (2011) Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (Musa sp.). J Food Compos Anal 1–10.

Survase SA, Bajaj IB, Singhal RS (2006) Biotechnological production of vitamins. Food Technol Biotechnol 44:381–396

Szűcs B, Simon M, Füleky G (2006) Anaerobic pre-treatment effects on the aerobic degradability of wastewater sludge. Proceedings of the Internationale Conference ORBIT 2006, Weimar, 13-15 September, 2006. Part 2, pp. pp 425-434.

Tambone F, Genevini P, D'Imporzano G, Adani F (2009) Assessing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW. Bioresour Technol 100:3140–3142

Tambone F, Scaglia B, D'Imporzano G, et al (2010) Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 5:577–583

Tamilselvi SM, Chinnadurai C, Ilamurugu K, et al (2015) Effect of long-term nutrient managements on biological and biochemical properties of semi-arid tropical Alfisol during

maize crop development stages. Ecol Indic 48:76–87

Tani M, Sakamoto N, Kishomoto T, Umetsu K (2006) Utilization of anaerobically digested slurry combined with other waste following application to agricultural land. Int Congr Ser 1293:331.

Teglia C, Tremier A, Martel JL (2011) Characterization of solid digestates: Part 2, assessment of the quality and suitability for composting of six digested products. Waste and Biomass Valorization 2:113–126

Tian X, Li S, Wang Z, et al (2003) Response of lettuce to different nitrogen forms. Ying yong sheng tai xue bao=. J Appl Ecol 14:377

Tiquia SM, Tam NFY, Hodgkiss IJ (1996) Effects of composting on phytotoxicity of spent pig-manure sawdust litter. Environ Pollut 93:249–256

Tiwari VN, Tiwari KN, Upadhyay RM (2000) Effect of crop residues and biogas slurry incorporation in wheat on yield and soil fertility. J Indian Soc Soil Sci 48:515–520

Trumbo P (2014) Pantothenic acid. In: Ross AC, Caballero B, Cousins RJ, et al., eds. Modern Nutrition in Health and Disease. 11th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2014:351-7.

Tshikalange B, Bello ZA, Ololade OO (2020) Comparative nutrient leaching capability of cattle dung biogas digestate and inorganic fertilizer under spinach cropping condition. Environ Sci Pollut Res 27:3237–3246

USDA (2015) National Nutrient Database for Standard Reference Release 28. USDA, Washington, D.C.

Valverde-Barrantes OJ, Freschet GT, Roumet C, Blackwood CB (2017) A worldview of root traits: the influence of ancestry, growth form, climate and mycorrhizal association on the

functional trait variation of fine-root tissues in seed plants. New Phytol 215:1562–1573

Wargovich MJ (2000) Anticancer Properties of Fruits and Vegetables

Warman PR, Havard KA (1997) Yield, vitamin and mineral contents of organically and conventionally grown carrots and cabbage. Agric Ecosyst Environ 61:155–162

Watson SA, Noggle. GR (1947) Effect of mineral deficiencies upon the synthesis of riboflavin and ascorbic acid by the oat plant. Plant Physiol 22:228–243

Webb AR, Hawkes FR (1985) Laboratory scale anaerobic digestion of poultry litter: Gas yield-loading rate relationships. Agric wastes 13:31–49

Weiland P (2010) Biogas production: current state and perspectives. Appl Microbiol Biotechnol 849–860

Wenke L, Du L, Yang Q (2009) Biogas slurry added amino acids decreased nitrate concentrations of lettuce in sand culture. Acta Agric Scand Sect B Soil Plant Sci 59:260–264

Wright AF, Bailey JS (2001) Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 dry combustion analyzer. Commun Soil Sci Plant Anal 32:3243–3258

Xu WH, Wang ZY, Quan YM, et al (2003a) Effect of application of biogas slurry on nitrate content and nutrition quality of lettuce and romaine lettuce. Rural Eco-Environment, 19:34–37

Xu WH, Wang ZY, Wang Q, et al (2003b) Effects of different biogas slurry on nutrient quality and nitrate content of lettuce. China biogas 21:11–13

Yalovsky S, Bloch D, Sorek N, Kost B (2008) Regulation of membrane trafficking, cytoskeleton dynamics, and cell polarity by ROP/RAC GTPases. Plant Physiol 147:1527–1543

You Q, Chen F, Wang X, et al (2012) Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. LWT-Food Sci Technol 46:164–168

Yu FB, Luo XP, Song CF, et al (2010) Concentrated biogas slurry enhanced soil fertility and tomato quality. Acta Agric Scand Sect B Soil Plant Sci 60:262–268

Zandvakili OR, Barker A V., Hashemi M, et al (2019) Comparisons of commercial organic and chemical fertilizer solutions on growth and composition of lettuce. J Plant Nutr 42:990–1000

Zandvakili OR, Ebrahimi E, Hashemi M, et al (2017) The potential of green manure mixtures to provide nutrients to a subsequent lettuce crop. Commun Soil Sci Plant Anal 48:2246–2255

Zhao X, Carey EE, Young JE, et al (2007) Influences of organic fertilization, high tunnel environment, and postharvest storage on phenolic compounds in lettuce. HortScience 42:71–76

Zuo J, Tang W, Xu Y (2015) Anti-Hepatitis B Virus Activity of Chlorogenic Acid and Its Related Compounds. In Coffee in health and disease prevention (pp. 607-613). Academic Press.