DEVELOPMENT OF A MANURE-BASED NUTRIENT SUPPLY FOR HYDROPONIC CROP PRODUCTION USING ION ACTIVITY MONITORING

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To my family:

This thesis is dedicated to you, for your love, support and encouragement throughout my education & life

Dear reader:

This is a non-fiction work with multiple plots

Abstract

With the increase in population and food demand, and, the limit of water and land available, novel methods are needed to produce food. Over the last decades, there has been a growth in controlled environments, especially greenhouses, and the use of hydroponic systems to produce food. In this context, the present thesis elaborates different approaches to grow food in a hydroponic system using organic fertilizers. To fulfill this goal, different animal manure extracts used in hydroponic system are examined and the nutrient solutions prepared are balanced to meet the plant needs.

The first study investigated different techniques to prepare a nutrient solution with cow, chicken and turkey manure. The objective of the study was to determine the impact of aerated manure extract on the growth of lettuce and kale under different manure concentrations. Nutrient analysis showed 29 to 79% higher concentration of NH_4 and higher total nitrogen in the manure extracts, which may have induced toxicity in plants. Plants that are susceptible to ammonium toxicity lack the ability to exclude ammonium through the plasma-membrane influx system and therefore, accumulate excessive amounts in the cytosol. Principal component analysis of the nutrient solutions identified six nutrient that needs to be monitored to maximize plant yield using manure extracts: NO_3, NH_4, Ca, Mg, Mn, and Na. The highest biomass was produced for both plants in the turkey extract at 50 g/L, while all plants died in the chicken extract at 50 g/L. In addition, besides ammonium and phosphorus, all other nutrients were below the suggested concentration required by plants to grow optimally.

The second study focused on a method to balance the nutrient solution made from animal manure. Specifically, powder and solid blast-furnace slag (90%) and Portland cement (10%) from Lafarge was added to chicken extract solution to balance the potassium and calcium levels. Animal manure is known to be nutrient deficient in K and Ca, while they contain Na which can be toxic to plants at high concentrations. The addition of Portland cement powder and cured cement blocks increased the Ca (from 38.8 to 57.4 to mg/L), K (from 93.4 to 121.2 mg/L) and Na (from 26.3 to 54.5 mg/L) content respectively in the solutions, without reaching toxic levels for Na. Healthy plants were grown in the manure and dry slag cement solution, however they were a fraction of the control treatment, 8% for kale and 19% for lettuce of total aboveground wet mass.

The third experiment focused on the reduction of ammonium and the increase of nitrate in the chicken manure extract. Aeration and the addition of molasses was used to promote the growth of beneficial microorganisms to mineralize the nutrients and make it available for the plants. Aeration of chicken extract solution promoted nitrification and resulted in a reduction of ammonium content by 62% within a period of 12 days. Molasses was added to promote denitrification and to control nitrate levels in the solution, however the nitrate levels were still minimal, below 10 mg/L, at the end of the experiment. During the experiment, an ion-selective electrode was used to monitor ammonium content over time.

In the fourth experiment, three methods of measurement for ammonium and nitrate were compared. This included the API water test kit, ion-selective electrodes (ISE) and the Lachat flow injection instrument. Currently, the Lachat spectrometry is the most commonly used method, however, ISEs allow for a continuous monitoring. The API test kit was not reliable even after the pre-treatment steps. ISE was the best method to measure NH_4, however a linear regression model was required to adjust ISE measurements. NO_3 in the manure extracts was low and difficult to measure with both API and ISE methods, however the ISE confirmed that the manure extracts were NO_3 deficient.

In the fifth study, a controlled area network binary unitary system (CAN bus) system was designed to monitor moisture and temperature levels in different hydroponic beds and to activate water pumps for irrigation purposes. The highest yield was measured in the timed treatment, where moisture varied from 88%-100%, with the lowest in the 25%-85% treatment. Individual plant wet and dry mass was monitored using a remote sensing instrument and confirmed at harvest. This system could be used in greenhouses with many crops, as the CAN bus is easy to setup and does not require to modify the software.

The sixth study was on the design and implementation of a bioreactor and a nutrient monitoring instrument using ion-selective electrodes in a research facility. Polar Eggs, a poultry farm located in Hay River, Northwest Territories of Canada, designed and created the PoutryPonics facility. This controlled environment facility was designed for experimenting food production and was created to address food insecurity in northern Canada. Within PoultryPonics, chickens were raised, and their manure was transformed to manure extracts for the use in hydroponic systems. The bioreactor was used to aerate the manure, to promote nitrification, and mix other organic residues to balance out the nutrient solution. The system was successfully implemented in the facility.

Résumé

Avec l’augmentation de la population et de la demande alimentaire, et la limite d’eau et de terres disponibles, de nouvelles méthodes sont nécessaires pour répondre à la demande en nourriture. Au cours des dernières décennies, il y a eu une croissance de l’agriculture dans des environnements contrôlés, en particulier des serres, et l'utilisation de systèmes hydroponiques pour la production de nourriture. Dans ce contexte, la présente thèse élabore différentes approches pour la culture de plantes dans un système hydroponique avec des engrais organiques. Pour atteindre cet objectif, différents extraits de fumier d'animaux ont été utilisés et examinés en tant qu'engrais organiques et les solutions préparés ont été équilibrés pour satisfaire les besoins nutritifs des plantes.

La première étude portait sur les techniques permettant de préparer une solution nutritive avec du fumier de vache, de poule et de dinde. L’objectif de l’étude était de déterminer l’impact de l’extrait de fumier aéré sur la croissance de la laitue et du chou frisé sous différentes concentrations de fumier. L'analyse des éléments nutritives a révélé une concentration de NH_4 supérieure de 29% à 79% et une teneur totale en azote plus élevée dans les extraits de fumier, ce qui aurait pu induire une toxicité chez les plantes. L’analyse en composantes principales des solutions nutritives a identifié six éléments nutritifs qui doivent être surveillés afin d’optimiser la pousse des plantes utilisant l’extrait de fumier: le NO_3, le NH_4, le Ca, le Mg, le Mn et le Na. La biomasse la plus élevée a été mesurée pour les deux plantes dans l'extrait de dinde à 50 g/L, tandis que toutes les plantes sont mortes dans l'extrait de poule à 50 g/L. De plus, mis à part l'ammonium et le phosphore, tous les autres éléments nutritifs étaient inférieurs à la concentration requise par les plantes pour une croissance optimale.

La seconde étude portait sur une méthode permettant d'équilibrer la solution nutritive à base de fumier animal. Spécifiquement, des scories de ciment solides et en poudre ont été ajoutées à une solution d'extrait de poule pour équilibrer les niveaux de potassium et de calcium. Le fumier animal est pauvre en nutriments K et en Ca, alors qu'il contient du Na qui peut être toxique pour les plantes à des concentrations élevées. L’addition de poudre de ciment de scories sèches et de blocs de ciment de scories durcis a augmenté les concentrations de Ca (de 38.8 à 57.4 mg/L), de K (de 93.4 à 121.2 mg/L) et de Na (de 26.3 à 54.5 mg/L) contenu dans les solutions, sans atteindre

des niveaux toxiques pour le Na. Des plantes saines ont été cultivées dans la solution de fumier et de ciment de scories durcis, mais elles représentaient une fraction de la masse totale hors sol humide du traitement de contrôle, 8% pour le chou frisé et 19% pour la laitue.

La troisième expérience portait sur la réduction de l'ammonium et l'augmentation des nitrates dans l'extrait de fumier de poule. L'aération et l'ajout de mélasse ont été utilisés pour favoriser la croissance de micro-organismes bénéfiques afin de minéraliser les nutriments et de les rendre disponibles pour les plantes. L'aération de la solution d'extrait de poule a favorisé la nitrification et a conduit à une réduction de la teneur en ammonium de 62% en l'espace de 12 jours. De la mélasse a été ajoutée pour favoriser la dénitrification et contrôler les niveaux de nitrate dans la solution. Toutefois, à la fin de l'expérience, les niveaux de nitrate étaient minimes, inférieurs à 10 mg/L. L'utilisation d'électrodes sélectives d'ions a permis de surveiller la teneur en ammonium à travers l'étude.

Dans la quatrième expérience, trois méthodes de mesure d'ammonium et de nitrate ont été comparées. Cela comprenait le kit de test d'eau API, les électrodes sélectives d'ions (ISE) et l'instrument d'injection de flux Lachat. Actuellement, la spectrométrie Lachat est la méthode la plus couramment utilisée. Cependant, les ISEs permettent une surveillance continue. Le kit de test API n'était pas fiable même après les étapes de prétraitement. L'ISE était la meilleure méthode pour mesurer NH_4, mais un modèle de régression linéaire était nécessaire pour ajuster les mesures. Le NO_3 dans les extraits de fumier était faible et difficile à mesurer avec les méthodes API et ISE, mais l'ISE a confirmé que les extraits de fumiers étaient déficients en NO_3.

Dans la cinquième étude, un système de CAN bus a été conçu pour surveiller les niveaux d'humidité et de température dans différents lits hydroponiques et pour activer les pompes à eau à des fins d'irrigation. Le rendement le plus élevé a été mesuré dans le traitement chronométré, où l'humidité variait de 88% à 100% et le plus faible dans le traitement de 25% à 85%. La masse sèche et humide des plantes individuelles a été mesurée à l'aide d'un instrument de télédétection et a été confirmée par pesée suite à la récolte. Ce système serait éventuellement utile dans les serres avec une grande variété de cultures, car le CAN bus est facile à installer et ne nécessite pas la reprogrammation du logiciel.

La sixième étude portait sur la conception et la mise en œuvre d'un bioréacteur et d'un instrument de surveillance des éléments nutritifs utilisant des électrodes sélectives d’ions dans une installation. L'installation PoultryPonics, un environnement contrôlé conçu pour la production alimentaire, a été créée pour lutter contre l'insécurité alimentaire dans le nord Canadien. À l’intérieur de l’installation, des poules ont été élevés et leur fumier a été transformé en extraits de fumier pour être utilisé dans des systèmes hydroponiques. Le bioréacteur a été utilisé pour aérer le fumier, favoriser la nitrification et mélanger d'autres résidus organiques pour équilibrer la solution nutritive. Le système a été implémenté avec succès dans l'installation.

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Contribution of Authors

In accordance with the guidelines of the Faculty of Graduate Studies and Research of McGill University "Guidelines for a Manuscript Based Thesis Preparation", the prepared manuscripts and contribution of authors are presented below.

Peter Tikasz is the principle author of this work, supervised by Professor Mark Lefsrud from the Department of Bioresource Engineering, McGill University, Montreal, Quebec, Canada. For Chapter 3 to 7, the entire construction, assembly and experimentations were performed in the Biomass Laboratory of the Bioresource Engineering Department. For Chapter 8, the construction of the bioreactor and assembly was completed at the PoultryPonics facility in Hay River, Northwest Territories, Canada.

Professor Mark Lefsrud, the supervisor and director of thesis, and Professor Viacheslav Adamchuk, supervising committee member, co-authored all manuscript and provided guidance in the planning and execution of the work as well as co-editing and reviewing of the manuscript.

Dr. Sarah MacPherson co-authored the second and third chapter, edited and proofread multiple sections of the manuscript.

Mrs. Yasmeen Hitti co-authored the fourth chapter and made valuable comments to improve the manuscript.

Mr. Roberto Buelvas co-authored the seventh chapter and made contribution by reviewing the article and helped running the experiments.

Mr. David Leroux co-authored the eight chapter, designed and helped setup the bioreactor at the PoultryPonics facility.

Details of the papers submitted are provided below:

1. Tikasz, P., MacPherson, S., Adamchuk, V. & Lefsrud, M. (2019), An update on organic fertilizers in hydroponics – a Canadian perspective. *Canadian Journal of Plant Science (Submitted).*

2. Tikasz, P., MacPherson, S., Adamchuk, V. & Lefsrud, M. (2019). Aerated chicken, cow, and turkey manure extracts differentially affect lettuce and kale yield in hydroponics. *International Journal of Recycling of Organic Waste in Agriculture (In press).*

3. Tikasz, P., Hitti, Y., Adamchuck, V. & Lefsrud, M. (2019), Effect of slag cement on calcium and potassium content used in hydroponic solutions made from chicken manure. *International Journal of Recycling of Organic Waste in Agriculture (Submitted).*

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6. Tikasz, P., Buelvas, R., Adamchuk, V. & Lefsrud, M. (2019). Development and monitoring of water stress on lettuce and kale grown in hydroponic systems with a CAN bus system. *Agriculture and Natural Resources (Submitted).*

7. Tikasz, P., Leroux, D., Adamchuk, V. & Lefsrud, M. (2019). Design of a bioreactor and nutrient monitoring instrument for food production in hydroponic systems located in northern Canada. *Horticultural Science & Biotechnology (Submitted).*

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1 INTRODUCTION

1.1 World population

The world population is approximately 7.7 billion, with 6 billion living in less developed countries, and is expected to reach between 9.4 and 10.1 billion by 2050 and between 9.4 and 12.7 billion by 2100 (Kumar & Cho, 2014; UN, 2005, 2019). Currently, about 793 million people are considered to be undernourished, which is a decrease of 167 million over the last decade, and 216 million since 1990-1992 (FAO, 2015). To meet the expected food needs of the growing population, a 70 to 100 % increase in food production is required (FAO, 2009; Pretty et al., 2010). With the current agricultural practices, an increase in food production will have a direct impact on arable land and water demand in the agricultural sector.

1.2 Food demand

Daily energy need will reach 3,050 kcal per person by 2050, an increase from 2,770 kcal per person in 2003-2005 (FAO, 2009). To meet human consumption needs, by 2050, projected demand for cereals will reach 3 billion tons, an increase from the current 2.1 billion tons, of which part of the demand will arise from the biofuel industry (FAO, 2009). One of the 2030 Sustainable Development Scenario (SDS) target is to use biofuel to meet 10% of the fuel demand. Yet, current levels are predicted to meet 3%, short of 7% (Sandquist, 2018). Meat production is expected to increase by 200 million tons over the same period, producing close to 465 million tons of meat and 1,043 million tons of milk (Pretty et al., 2010).

Arable land for agricultural purposes is expected to expand by 5% (about 70 million ha), yet most of the increase in production is anticipated to result from an increase in cropping intensity (FAO, 2009). As for freshwater use, current estimates revealed that about 70% of all freshwater withdrawals are used for food production and can go as high as 85% in some countries (Calzadilla et al., 2010; Pretty et al., 2010). The increase in agricultural output have often led to land and water systems degradation caused by management practices. It was calculated that even if the sustainable water supply would remain constant between 2000 and 2025, societal adaptations and water engineering will be necessary to meet the population's future water demand (Vörösmarty et al., 2000). Novel technologies, such as hydroponic systems, that allows an increase in food production while reducing land and water use are needed.

1.3 Food production with hydroponics systems

Hydroponic systems are a method to grow plants in a nutrient solution with the use of an artificial medium. Hydroponic systems present multiple advantages over traditional agriculture. These systems do not use soil and consequently poor soil fertility, soil erosion and compaction are not encountered. Pest control is easier and there is less use of pesticide and hydroponic systems allows to grow crops in locations that are unsuitable for growing crops due to toxic chemicals or heavy metals contaminating the soil (Lee & Lee, 2015; Surendran et al., 2016; Tomasi et al., 2015). Additionally, hydroponic systems use less water and have high water efficiency. Water and nutrients are not lost through leaching (Surendran et al., 2016). Concerning the yield, hydroponic systems have higher productivity per unit area than field grown crops and fruits and vegetables grown in hydroponic systems have higher nutritive values (Surendran et al., 2016; Suvo et al., 2017).

Parameters of hydroponic systems, such as the nutrient solution and the substrate used can be easily optimized for crop growth (Suvo et al., 2017). Hoagland solution, a nutrient solution commonly employed, was developed by Arnon and Hoagland in 1950 (Hoagland & Arnon, 1950). A substrate is necessary to support the plant. Good substrate should maintain moisture, be free of harmful microorganisms, with neutral pH, and not decomposed easily (Suvo et al., 2017). Some common substrates used are rockwool, jute fiber, cotton, coconut husk and sphagnum moss (Arancon et al., 2015). Current alternatives are being explored to replace inorganic fertilizers through the use of manure and other organic matter.

Hydroponic systems have limitations: there is a higher setup cost, rapid pathogen spread can occur and require a more specialized knowledge than traditional agriculture (Lee & Lee, 2015). Power failure can be detrimental for plants, as all devices regulating the hydroponic systems require electricity, such as pumps and lighting system. Finally, discharge of used nutrient solutions from these systems have a negative impact on the environment, although studies are already investigating the reuse of hydroponic waste solutions as an alternative for agriculture development and to control environmental pollution (Kim, Sudduth, et al., 2015).

1.4 Organic and inorganic fertilizers

Plants require 16 essential nutrients to optimize their growth (Table 1.1) (Gellings & Parmenter, 2016; Grusak et al., 2001; Taiz & Zeiger, 2002). Removal of mature plants deplete the soil of the nutrient content (Pretty et al., 2010). To replace the missing nutrients, fertilizers are added to the soil to maintain soil fertility and increase crop yield. These fertilizers can be organic or inorganic.

Table 1.1. List of essential plant nutrients (Gellings & Parmenter, 2016; Grusak et al., 2001; Taiz & Zeiger, 2002).

Element	Symbol	Form acquired	Class	Function
Nitrogen	N	NH_4^+, NO_3^-	Macronutrient, primary	Photosynthesis, metabolites
Phosphorus	P	$H_2PO_4^-$	Macronutrient, primary	Metabolites, structural, signaling
Potassium	K	K^+	Macronutrient, primary	Osmotic, electrochemical, metabolism
Calcium	Ca	Ca^{2+}	Macronutrient, secondary	Structural, signaling
Magnesium	Mg	Mg^{2+}	Macronutrient, secondary	Photosynthesis, metabolism
Sulphur	S	SO_4^{2-}	Macronutrient, secondary	Metabolites, structural
Boron	B	$B(HO)_3$	Micronutrient	Structural
Chlorine	Cl	Cl^-	Micronutrient	Osmotic, electrochemical
Copper	Cu	Cu^+, Cu^{2+},	Micronutrient	Redox

		Cu chelates		
Iron	Fe	Fe^{2+}, Fe^{3+} chelates	Micronutrient	Photosynthesis, redox
Manganese	Mn	Mn^{2+}, Mn chelates	Micronutrient	Redox
Molybdenum	Mo	MoO_4^{2-}	Micronutrient	Redox
Nickel	Ni	Ni^{2+}, Ni chelates	Micronutrient	Urease
Sodium	Na	Na^{+}	Micronutrient	Osmotic, electrochemical, redox
Zinc	Zn	Zn^{2+}, Zn chelates	Micronutrient	Redox
Aluminum	Al	Al^{3+}	Beneficial	Structural
Cobalt	Co	Co^{2+}	Beneficial	Symbioses
Silicon	Si	$Si(OH)_4$	Beneficial	Structural

Inorganic fertilizers are commercially manufactured chemicals with various nutrient compositions made for specific crops and soil types (Gellings & Parmenter, 2016). Fertilizers are differentiated by their nitrogen-phosphate-potash levels, where 44% of phosphorus is from phosphate (P_2O_5) and 83% of potassium is from potash (K_2O).

Organic fertilizers contain beneficial microorganisms and can be produced from manure, crop residues, compost and sewage sludge. Presence of microorganisms help decompose organic material, increase nutrient availability for plants, improve soil water-holding capacity, enhance aeration and improve soil aggregation (Gellings & Parmenter, 2016; Joshi et al., 2015). Organic fertilizers are considered more environmentally friendly as compared to mineral fertilizers, although they may contain elevated concentrations of toxic metals if they are derived from industrial waste, such as Fe, Zn, Cu, Cr and Pb, and, have high energy requirements (Goswami et al., 2014). Transportation and application costs are often higher since they have less nutrient content per unit mass and therefore inorganic fertilizers are more cost-effective than organic fertilizers if the latter is applied at a greater distance from the production location (Gellings & Parmenter, 2016).

1.5 Organic food production

The relatively high costs of inorganic fertilizers and policies aiming at reducing the use of chemicals in agriculture are promoting the movement towards organic fertilizers in both traditional agriculture and hydroponic systems (On et al., 2015). This facilitated the use and study of compost teas and vermicompost, an alternative to agrochemicals, which are aerated or nonaerated water extracts from composts (Kawamura-Aoyama et al., 2014; Pane et al., 2016). Preliminary studies have shown that compost tea can stimulate plant root and vegetative growth as well as increase crop yield and quality (Abul-Soud et al., 2016; On et al., 2015; Pane et al., 2016). Abundant studies have focused on the beneficial bacteria and fungi in different compost teas and vermicompost including *Pseudomonas*, *Bacillus* and *Streptomyces* (Lee & Lee, 2015). In one study, *Pseudomonas* and *Bacillus* decreased the infection by *Pythium* when applied to chrysanthemums (Lee & Lee, 2015). Compost teas can be made with compost or liquid extracts of manures while vermicompost are stabilized organic matter decomposed by earthworms, a non-thermophilic process (Abul-Soud et al., 2016). Manure extracts, a nutrient solution prepared like the compost teas without the composting step, are being investigated as a potential substitute to standard fertilizers (Martin, 2014).

Ongoing studies are investigating the effect of microorganisms to promote plant growth in hydroponic systems. Goat, chicken and beef manure have been used to replace inorganic fertilizers in hydroponic systems, yet the outcomes do not always converge. This difference was caused by the methodology used, analysis performed and results obtained (Table 2.1) (Mowa, 2015). Livestock and poultry manure management employed to minimize the risk of polluting surface waters, contaminating wells or discharging ammonia in the air increases farmers operating expenses (Liedl et al., 2004). Reusing manure from the poultry industry for hydroponic growth would save farmers millions of dollars as treatments are required before manure disposal (Liedl et al., 2004). However, studies are still required to determine the optimum manure concentration and monitoring essential nutrients in manure extracts could improve the solution preparation and would accelerate their implementation in hydroponic systems.

1.6 Automation of hydroponic systems

Sensors are being combined with hydroponic systems to monitor the nutrient content of hydroponic solutions, identify signs of crop toxicity or water stress, as well as to reduce human labor and production costs (Arefi et al., 2011; Golzarian et al., 2007).

Most hydroponic systems use timers to automate water addition. However, optimal irrigation schedules are still being debated, and often it is up to the grower's personal experience to determine the irrigation schedule of a plant (Möller et al., 2007; Schröder & Lieth, 2002). Machine vision and image analysis can be used to determine crop water stress levels based on leaf turgidity, leaf movement and changes in leaf angle (Kacira et al., 2002). Another method employes infrared thermometer to measure canopy temperature and combine the records with vapor pressure deficit to determine crop water stress index (Irmak et al., 2000). However, determining crop water stress index in a greenhouse with partial canopy cover and low plant population early after plants have been transplanted can lead to water stress overestimation and plant's overirrigation, as a result, can occur.

Most hydroponic solutions are prepared from stock solutions. Electrical conductivity (EC) and pH sensors are used to monitor the nutrient content. Although EC sensors are inexpensive, they do not provide an accurate information on the concentration of individual ions (Bamsey et al., 2012; Kim et al., 2013). Ion-selective electrodes (ISE) are a suitable instruments to measure macronutrients in closed hydroponic systems which use manure extracts or reuse drainage solutions (Hashimoto et al., 1988; Morimoto et al., 1991). Conversely, ISE probes allow for specific ion monitoring in a solution and can provide information to determine missing nutrients that can then be used to rebalance the solution. Employing ISE probes in hydroponic systems would allow for a fast and automatic sampling and could be useful in monitoring manure extracts composition during their preparation and plant growth.

1.7 **Objectives**

Current trends show an increase in the price of inorganic fertilizers with a continuous increase in demand for such fertilizers over time, a transition towards greenhouse plant production with hydroponic systems, and a growth in the organic product market (Gellings & Parmenter, 2016; Inácio et al., 2015; Statistics Canada, 2010). In Canada, lettuce and kale were amongst the top five vegetable imports in 2016, representing $447 million and $374 million in annual sales, respectively (Agriculture and Agri-Food Canada, 2017). During the same period, lettuce grown in Canada generated $112 million in revenue. There is therefore a need to develop hydroponic systems employing organic fertilizers adapted to both existing and emerging greenhouse markets. Preliminary tests indicate that organic fertilizers can be used in hydroponic systems. It is therefore of interest to develop a methodology for organic fertilizer preparation and real-time nutrient monitoring for hydroponic systems. It was hypothesized that the source of manure extract fertilizers and preparation methods will impact the nutrient composition of the solutions. Moreover, manure extracts with unknown nutrient levels should lead to unbalanced nutrient solutions that could be detrimental for plant growth. Through the measurement and analysis of the organic fertilizer used in hydroponic systems, we set out to monitor and identify key nutrients. The plants' response to different manure-based fertilizers with additives would allow to modify the nutrient composition of the solutions to improve plant growth. It was hypothesized that automating water supply and monitoring macronutrients with ion-selective electrodes in hydroponic systems would provide necessary information to balance the nutrient levels of manure-based fertilizers, improve plant growth environment, and production.

The overall aim of this thesis was to develop and optimize a hydroponic crop production nutrient supply system that uses animal manure as a source of fertilizer, to automate water supply, and to implement macronutrient monitoring using ion-selective electrodes. The specific objectives of this project are focused on the:

1. Design of a methodology to prepare animal manure in an ebb and flow hydroponic systems for lettuce and kale growth using chicken, cow and turkey manure.
2. Balance chicken manure extract nutrition levels used for plant growth in hydroponic solution with the addition of solid slag cement.

3. Control ammonium and nitrate content in the chicken manure extract solution using molasses and aeration.
4. Compare ion-selective electrodes and API water test kit to the Lachat flow injection method to measure nutrients in hydroponic systems using manure extracts.
5. Automate irrigation of hydroponic systems with a CAN bus system and collect moisture and temperature data from the rockwool.
6. Design a nutrient monitoring instrument with ion-selective electrodes that can be incorporated in a bioreactor to prepare manure-based fertilizer for hydroponic systems.

Connecting Text

Chapter 1 highlighted the use case of hydroponic systems to meet global food demand. The transition towards organic fertilizer and the automation of hydroponics systems were presented as the primary research umbrella under which the objectives were divided.

Chapter 2 reviews the current status of controlled environment agriculture, the use of organic fertilizers and the automation of hydroponic systems. The first section assesses the demand for inorganic fertilizers and the growth of greenhouses in North America. The evolution of organic market over the last decade and the different types of organic fertilizers are presented, including; compost, compost teas, vermicompost, and vermiwash. The chapter further examines the regulations around organic waste disposals and the presence of pathogens and harmful chemicals that could be a potential threat to consumers. Afterward, the automation of nutrient monitoring with ion-selective electrodes is presented with the automation of irrigation systems. The information provided gives a basic understanding of the current knowledge on organic hydroponic practices with its limitation and supports the need for further research for proper organic fertilizer preparation, nutrient monitoring, and irrigation automation.

Chapter 2 has been submitted for publication on March 20th, 2019:

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2 REVIEW: COMPOST TEAS AND HYDROPONIC SYSTEMS

2.1 Global fertilizer market

Nitrogen (N), phosphate (PO_4^{3-}), and potash (K_2O) are the main nutrients for plant growth and the most frequently used fertilizers. Their global use is 80 times higher than it was nearly 100 years ago (FAO, 2017). In 2014, global fertilizer sales were estimated at USD $172 billion, and prices have steadily increased over the last 10 years, following a peak in early 2008 (Gellings & Parmenter, 2016; Heffer & Prudhomme, 2014). In the 12 months prior to April 2008, the price of nitrogen increased by 32%, phosphate increased by 93%, and potash increased by 100% (USDA, 2013). This price surge was a consequence of low fertilizer inventories, the inability of the fertilizer industry to adjust production levels, and a strong domestic and global demand for fertilizers (Huang et al., 2009). In 2013, the price of fertilizer surpassed those of 2008 and kept on increasing (USDA, 2013).

To meet current agricultural demands, the construction cost of new mines and new fertilizer production facilities is estimated at USD $83 billion over the next few years (Heffer & Prudhomme, 2014). Out of the 285.15 tons of fertilizer available in 2016, over 186 million tons were applied, and the demand is forecast to grow by 1.3% on average annually until 2023 (FAO, 2017; IFA, 2018). In North America, over 14,000 thousand tons N, 5,000 thousand tons P in form of P_2O_5, and 4,900 thousand tons K in form of K_2O are needed annually (FAO, 2017). The observed increase in fertilizer use over time is the result of increased fertilizer applications rates to improve yield and mitigate nutrient deficiency in depleted soils caused by continuous harvesting of crops (Brentrup & Palliere, 2008).

In the North American agricultural industry, 31% of energy consumed goes towards inorganic fertilizer manufacturing (McLaughlin et al., 2000). In the US, natural gas supplies between 70 to 80% of all energy to fertilizer production (Gellings & Parmenter, 2016). On a global scale, fertilizer manufacturing consumes approximately 1.2% of the world's energy and it is responsible for 1.2% of total greenhouse gas emissions, of which 0.3% is pure CO_2, 0.3% is N_2O, and 0.6% is flue CO_2 gas (Kongshaug, 1998). In Canada, chemical fertilizer production with potash used 31.9 PJ in 2016 (NRS, 2016). This industry produced over 25 million metric tons of fertilizer annually of which 20 million is exported and contributes CAD $12 billion to Canada's

GDP. Recently, in 2010, the Canadian Fertilizer Institute (CFI) has committed CAD $400,000 to improving manufacturing efficiency (CFI, 2012).

Technological advances within the fertilizer manufacturing industry could reduce global energy consumption by as much as 40%, and greenhouse gas emission could be reduced by 60% (Brentrup & Palliere, 2008). The use of organic fertilizers is another feasible and sustainable alternative. For instance, it is estimated that close to 4.6 PJ energy could be saved annually in North America if inorganic fertilizers are substituted with manure for grain corn production (McLaughlin et al., 2000). A study estimated that conversion of only organic agriculture would reduce 38% of base-line energy consumption in Croatia (Znaor et al., 2007). In Canada, organic cultivation of wheat-pea-wheat-flax crop rotation required 50% less energy and was more energy efficient (energy produced / energy used) than conventional management even if the energy output was 30% lower in organic productions (Hoeppner et al., 2006). However, proper cost analysis is required as organic agriculture tends to have lower yield than traditional agriculture even if the energy savings is greater as production and transportation costs are minimal (FAO, 2007).

This update reviews compost, compost teas, and vermicompost production, as well as nutrient composition, and the feasibility of applying these organic fertilizers in hydroponic food production systems. Preparation methods of organic fertilizers are summarized as well as potential environmental and health impacts are presented.

2.1.1 Organic fertilizers

The growing organic food market, high costs of inorganic fertilizers, and policies aimed at reducing chemical use in agriculture are promoting the movement towards organic fertilizers (On et al., 2015). In 2012, the organic food market was valued at USD $63.8 billion in the US alone, and at CAD $5.4 billion in Canada (Inácio et al., 2015). Consumers show a disposition towards organic food, as it is associated with health, high quality products, and environmentally-friendly agricultural practices (Inácio et al., 2015; Peiris & Weerakkody, 2015). Adding the organic label to food products can increase retail prices from 15% to 50% (Brentlinger, 2005).

2.1.2 Fruit and vegetable market in Canada

Fruit and vegetable production in Canada are lucrative sectors in both domestic and global markets, and novel technologies facilitate sector growth. From 2015 to 2016, the farm gate value of fruit and vegetable products increased in Canada by 7.4%, reaching CAD $1.2 billion (Agriculture and Agri-Food Canada, 2017). Products such as carrots, tomatoes, lettuce, dry onions, sweet corn, broccoli, and peppers represented more than 50% of the total field-grown vegetables (Agriculture and Agri-Food Canada, 2017). Carrot sales represented CAD $129.3 million, while tomatoes represented CAD $110.1 million and lettuce represented CAD $78.4 million (Agriculture and Agri-Food Canada, 2017). The quantity of fruit and vegetables produced has increased steadily over the past five years, and the export market grew from CAD $0.5 billion to CAD $0.8 billion in revenue from between 2013 and 2017 (Agriculture and Agri-Food Canada, 2017).

The observed increase in fruit and vegetable production may be partly explained by the implementation of greenhouses and hydroponic systems (Statistics Canada, 2010) that allow for multiple cropping each year (Sheridan et al., 2016). From the vegetables grown, 97.8% were exported to U.S., 1.0% to Netherlands, 0.5% to Japan and 0.7 to other countries (Agriculture and Agri-Food Canada, 2017). In 2016, the greenhouse industry employed over 4,600 seasonal and 6,500 permanent employees.

In parallel with the increase of the organic market, there is an increase in composting. On average, each Canadian generates about 383 kg of solid waste, however only one fifth is recycled (Statistics Canada, 2005). In 2002, 1.2 tonnes of organic waste was composted by centralized compositing facilities in Canada (Statistics Canada, 2005). Between 2000-2010, composting of household food and yard waste increased by 125% (Giroux, 2014). Compost can be re-used as a source of nutrient in hydroponic systems (Jarecki et al., 2005). Nutrient content in compost teas used in hydroponic systems needs to be assessed to make sure appropriate levels of nutrients are present to promote plant growth (Hernández et al., 2014). Animal manure, another type of solid waste, can also be used as a source of nutrients in hydroponic systems.

The total amount of manure produced by 100 million cattle and hog in the U.S. is estimated between 1.23 billion tons and 116.65 million tons annually respectively (Fayer & Trout, 2005;

Pachepsky et al., 2006). One cow can produce an average of 1,300 kg of manure per year (Mitchell, 1997). In comparison, 1,000 egg-laying or meat chickens excrete 120 kg and 80 kg daily (43,800 kg and 29,200 kg annually), respectively (FAO, 2013). Reusing industrial poultry manure for hydroponic growth could save farmers millions of dollars, as treatments of wastes are required before manure disposal (Liedl et al., 2004). To be economically viable, however, it is estimated that broiler compost should be applied within 15 miles of the broiler house, as transportation fuel will require more energy and the process will no longer be cost-effective (Gellings & Parmenter, 2016).

2.1.3 Compost tea

Organic fertilizers are compost, manure, and vermicompost, as well as their derivatives, including aerated or non-aerated water extracts referred to as compost teas (Kawamura-Aoyama et al., 2014; Pane et al., 2016). Fermentation and application parameters influence compost tea production and efficacy as an organic fertilizer. However, the optimal method for organic fertilizer production had been debated (Ingham, 2002; Scheuerell & Mahaffee, 2002). Fermentation parameters include the compost source and characteristics, the fermentation vessel, water used, type(s) of fermentation nutrients added to the compost tea, oxygen content, and duration of the fermentation process (Scheuerell & Mahaffee, 2002). Age, moisture content, the presence of earthworms and microbes, available nutrients, and quantity of the manure or compost also influences compost tea composition (Tables 2.1, 2.2, 2.3 and 2.4) (Pant et al., 2012; Scheuerell & Mahaffee, 2002). Application parameters include a filtration step (and material used), dilution ratio, any additional nutrients or steps taken to stabilize nutrients, application equipment, and the application process itself (Scheuerell & Mahaffee, 2002).

Composting is a thermophilic process where organic matter is mineralized via microorganisms (Pant et al., 2012). Mature compost has higher mineral availability and lower release of heavy metals with phytotoxic organic acids than immature compost (Pant et al., 2012). Higher mineral content in compost results in composts teas with a greater mineral content (Pant et al., 2012). Further to this, compost teas provide microbial biomass, organic acids, and mineral nutrients to the plant (Pant et al., 2012). Notably, compost teas can stimulate plant root and vegetative growth, increase crop yield and quality, and prevent the development of plant diseases (Abul-Soud et al., 2016; On et al., 2015; Pane et al., 2016). For example, it has been reported that compost teas increased growth and mineral nutrient content of pak choi (*Brassica rapa* B.) (Pant et al., 2012), while application of compost teas on agricultural soil increased tomato (*Solanum lycopersicum* L.) yield over time (Pane et al., 2016).

Manure can be detrimental for plant growth, even at low concentrations. Compost teas derived from manure have been investigated, and studies reporting the effects of different composts on biomass and nutrient levels are summarized in Table 2.1. Notably. Atiyeh et al. (2000) showed that amending soil with 4% chicken manure compost high in ammonium burned the plant roots and killed most raspberry plants.

2.1.4 Vermicompost

Vermicompost is stabilized organic matter that is digested and excreted by earthworms via a non-thermophilic process (Abul-Soud et al., 2016). Specifically, 73% of the earthworm's gut is filled with *Vibrio* sp., a facultative anaerobic bacterium that is responsible for the degradation of ingested food, allowing the gut to act as a bioreactor (Manyuchi et al., 2012). Earthworms produce vermicasts after digestion is complete and addition of earthworms to compost can reduce composting time by 25% (Sundaravadivelan et al., 2011).

Earthworms reduce composting time (Sundaravadivelan et al., 2011). Most commonly used earthworms for vermicompost are the red worm or red wriggler (*Eisenia foetida* or *Eisenia Andrei*), the red earthworm (*Lumbricus rubellus*), and the European nightcrawler (*Eisenia hortensis*) (Allahyari et al., 2014; Manyuchi et al., 2012). *Eisenia foetida* and *Lumbricus rubellus* have adapted to environments high in organic matter, including rotting vegetation, compost, and manure (Ansari & Sukhraj, 2010). A pH of 5.0 to 9.0 is optimal to maintain earthworm growth (Manyuchi et al., 2012).

Vermiwash is a vermicompost by-product. It is the fluid collected from vermicompost tanks, containing earthworms' secretions with micronutrients from soil and digested organic matter (Sundaravadivelan et al., 2011). Both vermicompost and vermiwash are rich in vitamins, antibiotics, microelements, minerals, and enzymes (Edwards & Burrows, 1988). In addition, the nutrient content of the vermicompost and vermiwash is influenced by the earthworm species. Sundaravadivelan et al., (2011) showed that *E. eugenia* earthworms have greater waste mineralization potential than *L. mauritii* and produce an output higher in nutrient levels.

The compost source (fruit, vegetables, manure, and sewage sludge) will also influence vermicompost nutrient composition (Table 2.1) (Sundaravadivelan et al., 2011). It is important to note that vermicompost has higher nutrient content (nitrate, calcium, phosphorus, and potassium) and a more diverse microbial population than regular compost (Tables 2.2, 2.3 and 2.4) (Pant et al., 2012).

Addition of different organic materials can affect vermicompost quality. Cow manure is often added to increase micro- and macronutrients in vermicompost (Muthukumaravel et al., 2008; Palanichamy et al., 2011), as it is rich in bacteria that can accelerate the composting process (Palanichamy et al., 2011). A slow growth rate observed for plants cultivated with vermicompost may be due to a higher salt content or excess nutrients. Furthermore, the presence of auxins, cytokinins and humic acids produced by microorganisms present in vermicompost can reduce the rate of plant development, yet at lower concentrations, the growth rate could increase (Arancon et al., 2008).

Vermicompost derived from manure can increase rates of germination, growth, and flowering (Arancon et al., 2008; Quaik et al., 2012). The addition of vermiwash solution to Indian borage *(Plectranthus amboinicus)* increased germination percentage to 80%, 30% over mature compost with a germination rate of 50% (Quaik et al., 2012). A similar study with petunia (*Petunia* sp.) seeds resulted in a faster germination rate, as well as higher shoot and root dry weights when vermicompost was added to soil mixture (Arancon et al., 2008). Contrarily, okra plants exhibited stunted growth when vermiwash was applied (Palanichamy, 2011), yet higher fat and protein content was measured in the okra fruit (Ansari & Sukhraj, 2010). It is possible that high salinity in the vermiwash limited okra growth, as this plant is detrimentally affected by salinity above 4 mS/cm (Palanichamy et al., 2011). In the radish (*Raphanus sativus* L.) plant, high concentrations of vermicompost can also inhibit germination and plant growth, and the study authors concluded that vermiwash should be diluted in order to optimize plant growth (Gutiérrez-Miceli et al., 2011).

Table 2.1. Summary of reported preparation methods for compost, compost tea, vermicompost, and vermiwash.

Experiment	Input	Hydro ponic	Experimental plant	Earthworm	Animal manure	Compost source and dilution	Preparation time	Dilution used	Comments	Reference
Vermiwash	Manure and plant	--	Okra (*Abelmoschus esculentus*)	*Eisenia fetida*	Cow	Loamy soil and dried grass	60 days for vermicompost process	--	Higher fat and protein content in okra; improved soil biochemical characteristics (soil micronutrients)	(Ansari & Sukhraj, 2010)
Vermicompost	Manure and plant	--	Okra (*Abelmoschus esculentus*)	*Eisenia fetida*	Cow	Loamy soil and dried grass	60 days for vermicompost process	--		
Vermicompost	Manure and plant	--	Petunia (*Petunia* sp. (var. Dreams Neon Rose F1)	*Eisenia fetida*	Cow	Pre-processed cattle vermicompost	79 days	--	Used as a substrate; presence of vermicompost accelerated germination; lowest germination in substrate mixed with cow vermicompost with high manure content; shoot dry weight and root dry weight of petunia negatively affected by higher vermicompost substitution rates; increased germination, increased dry shoot and root weight, and number of flowers obtained with half-half substitution Vermicompost and MM360 substrate mixture	(Arancon et al., 2008)
Vermicompost	Earthworms	--	Petunia (*Petunia* sp. (var. Dreams Neon Rose F1)	*Eisenia fetida*	--	Food waste	79 days	--		
Vermicompost	Earthworms	--	Petunia (*Petunia* sp. (var. Dreams Neon Rose F1)	*Eisenia fetida*	--	Paper waste	79 days	--		
Vermicompost	Manure	--	Tomato (NA)	*Eisenia fetida* (Savigny)	Pig manure	--	--	--	Humic acids extracted from vermicompost increased tomato and cucumber growth	(Atiyeh et al., 2002)
Vermiwash	Manure	--	French dwarf bean (*Phaseolus vulgaris* L.)	*Eisenia fetida*	Cow	25 g earthworms / kg cow manure	2 months	100 kg of vermicompost flushed with 50 L water	Vermiwash increased plant height, length of pods, and number of pods; vermicompost with vermiwash may be used as fertilizer for bean cultivation	(Ayyobi et al., 2014)
Manure	Manure	--	--	--	Cow	Dilution 1: 10	--	--	Ammonium concentrations within recommended range to be used as fertilizer; nitrate levels were highest in vermicompost and lowest in compost tea.; vermicompost had highest phosphorus content and potassium content	(El-Haddad et al., 2014)
Compost	Plant	--	--	--	--	Rice straw; dilution 1: 10	Time undisclosed	--		
Compost	Plant	--	--	--	--	Rice straw; dilution 1: 10	Time undisclosed	--		
Vermicompost	Manure and plant	--	--	*Eisenia fetida*	Cow	Rice straw; dilution 1: 10	Time undisclosed	----		

Experiment	Input	Hydro ponic	Experimental plant	Earthworm	Animal manure	Compost source and dilution	Preparation time	Dilution used	Comments	Reference
Vermicompost	Manure	--	Radish (*Raphanus sativus* L.)	*NA*	Sheep manure	--	--	--	At a high application rate, vermicompost inhibited germination; likely caused by high salt concentration, pH, or humic and fluvic acids	(Gutiérrez-Miceli et al., 2011)
Vermiwash	Manure	--	Radish (*Raphanus sativus* L.)	*NA*	Sheep manure	--	--	--		
Compost tea	Manure	--	Tomato (*Lycopersicon esculentum* L.)	--	Chicken	--	90-day old compost	5:1 (v/L) water: compost	ACT; chicken manure increased disease suppression and increased fruit yield	(Haggag & Saber, 2007)
Compost tea	Manure	--	Tomato (*Lycopersicon esculentum* L.)	--	Chicken	--	90-day old compost	5:1 (v/L) water: compost	NCT; chicken manure increased disease suppression and increased fruit yield	
Compost tea	Manure	Yes	Tomato (*Lycopersicum esculentum* Mill. cv. "Optima")	--	Sheep and goat manure	--	--	--	Compost had no effect on tomato yield due to low nutrient content in the solution	(Hernández et al., 2014)
Compost tea	Manure and plant	Yes	Tomato (*Lycopersicum esculentum* Mill. cv. "Optima")	--	Cow manure + alperujo + olive prunings	--	--	--		
Compost tea	Plant	Yes	Tomato (*Lycopersicon esculentum* Mill. 'Roma VF') and marigold (*Tagetes erecta* L. 'Crackerjack')	--	--	Spent mushroom	--	--	Highest yield in Hoagland solution; pH and EC adjusted at beginning of experiment	(Jarecki et al., 2005)
Compost tea	Plant	Yes	Tomato (*Lycopersicon esculentum* Mill. 'Roma VF') and marigold (*Tagetes erecta* L. 'Crackerjack')	--	--	Pond-collected runoff	--	--	pH and EC adjusted at beginning of experiment	
Fertilizer	Plant	Yes	Tomato (*Lycopersicon esculentum* Mill. 'Roma VF') and marigold (*Tagetes erecta* L. 'Crackerjack')	--	--	Pond-collected runoff	--	--	pH and EC unadjusted at beginning of experiment; full-strength Hoagland	
Fertilizer	Plant	Yes	Tomato (*Lycopersicon esculentum* Mill. 'Roma VF') and	--	--	Pond-collected runoff	--	--	pH and EC adjusted at beginning of experiment; half-strength Hoagland	

Experiment	Input	Hydro ponic	Experimental plant	Earthworm	Animal manure	Compost source and dilution	Preparation time	Dilution used	Comments	Reference
			marigold (*Tagetes erecta* L. 'Crackerjack')							
Compost	Plant	--	Oilseed rape (*Brassica napus* L.)	--	--	Mature green waste	Composted for 12 weeks and matured for 9 months	--	Total plant weight was only enhanced by compost tea at lowest dilution ratio	(Keeling et al., 2003)
Compost	Plant	--	Oilseed rape (*Brassica napus* L.)	--	--	Mature green waste	Composted for 12 weeks and matured for 3 months	--		
Compost tea	Plant	Yes	Oilseed rape (*Brassica napus* L.)	--	--	Mature green waste	Composted for 12 weeks and matured for 3 months	Compost: water 1: 3		
Fertilizer	Plant	--	Oilseed rape (*Brassica napus* L.)	--	--	Mature green waste	Composted for 12 weeks and matured for 3 months	--		
Vermiwash	Plant	--	Bhut Jolokia (*Capsicum assamicum*)	*Eisenia fetida* (Savigny)	--	Vegetable waste: paddy straw: water hyacinth; 1:1:1	--	--	Vermiwash foliar spray modifies crop response to mycorrhizal fungi with respect to growth and nutrient utilization	(Khan et al., 2014)
Compost tea	Manure	--	Tomato (cultivar Bush Beefsteak)	--	Chicken	--	Compost tea preparation 14 days	Water: compost 5:1 (v/L)	NCT were able to control tomato ray mold but not powdery mildew	(Koné et al., 2010)
Compost tea	Manure	--	Tomato (cultivar Bush Beefsteak)	--	Bovine	--	Compost preparation Compost tea preparation 14 days	Water: compost 5:1 (v/L)		
Compost tea	Manure	--	Tomato (cultivar Bush Beefsteak)	--	Sheep	--	compost preparation NA; compost tea preparation 14 days	Water: compost 5:1 (v/L)		
Compost tea	Manure and plant	--	--	--	Fish waste	Seaweed (*Laminaria* sp. and *Cystoseira* sp.) and pine bark	4 months composting, left to mature 2 months	Aqueous extract 1:5	Potential use asorganic fertilizer made from fisheries by-products	(López-Mosquera et al., 2011)
Compost	Manure and plant	--	--	--	Fish waste	Seaweed (*Laminaria* sp. and *Cystoseira* sp) and pine bark	4 months composting, left to mature 2 months	--		

Experiment	Input	Hydro ponic	Experimental plant	Earthworm	Animal manure	Compost source and dilution	Preparation time	Dilution used	Comments	Reference
Vermicompost	Manure and plant	--	--	*Eisenia fetida*	Cow	Food waste, corn pulp: cow manure ratio of 6: 1	30 days for vermicompost process	--	Vermicompost became more acidic, due to nitrogenous waste excreted by earthworms	(Manyuchi et al., 2012)
Compost tea	Manure and plant	--	--	--	Chicken	Straw	Period of 98 to 114 days	--	Greatest nitrogen lost, caused by gaseous emissions in the form of NH_3 mostly and smaller amounts of NO_x	(Martins & Dewes, 1992)
Compost tea	Manure and plant	--	--	--	Cow	Straw	Period of 98 to 114 days	--		
Compost tea	Manure and plant	--	--	--	Pig	Straw	Period of 98 to 114 days	--		
Compost tea	Manure and plant	--	--	--	Mix of pig, cow and chicken	Straw	Period of 98 to 114 days	--		
Fertilizer	Plant	Yes	Forsythia (*Forsythia 3 intermedia* 'Lynwood'), weigela (*Weigela florida* 'Red Prince'), creeping bentgrass (*Agrostis palustris Huds.*), and Kentucky bluegrass (*Poa pratensis* L.)	--	--	--	--	Half-strength Hoagland solution	Higher yield of turfgrass in inorganic fertilizers	(Michitsch et al., 2007)
Fertilizer	Plant	Yes	Forsythia (*Forsythia 3 intermedia* 'Lynwood') and weigela (*Weigela florida* 'Red Prince'), and creeping bentgrass (*Agrostis palustris Huds.*) and Kentucky bluegrass (*Poa pratensis* L.)	--	--	--	--	Plant product fertilizer		
Vermicompost	Manure	--	--	*Eisenia fetida*	Cow	--	13 weeks	Water: solid 5: 1	Vermicompost reduced EC, neutralized pH, and reduced harmful chemical contaminant concentrations	(Mitchell, 1997)

Experiment	Input	Hydro ponic	Experimental plant	Earthworm	Animal manure	Compost source and dilution	Preparation time	Dilution used	Comments	Reference
Vermiwash	Plant	--	Corn (*Zea mays* L.)	--	--	--	--	--	Vermiwash application increased seed production of corn	(More et al., 2013)
Compost	Manure and plant	--	--	--	Cow	Bottom ash: cow manure (50:50, v/v)	3 weeks	--	Lower cow manure content mixed with bottom ash may be appropriate as a soil amendment	(Mukhtar et al., 2003)
Compost tea	Manure and plant	--	--	--	Cow	Bottom ash: cow manure (50:50, v/v)	3 weeks	--		
Vermicompost	Earthworms	--	--	*Megascolex mauritii*	--	Soil	60 days for vermicompost process	--	Compost with highest NPK values obtained when vegetable waste was mixed with cow manure	(Muthukumaravel et al., 2008)
Vermicompost	Manure and plant	--	--	*Megascolex mauritii*	Cow	Soil	60 days for vermicompost process	--		
Vermicompost	Earthworms	--	--	*Megascolex mauritii*	--	Vegetable waste	60 days for vermicompost process	--		
Vermicompost	Manure and plant	--	--	*Megascolex mauritii*	Cow	Soil and Vegetable waste	60 days for vermicompost process	--		
Vermiwash	Manure	--	--	*Eisenia foetida*	Sheep	--	3 weeks	NA	Total N, P, K and Ca significantly decrease with earthworm activity and can be used as biofertilizer for organic ferming	(Nath et al., 2009)
Vermiwash	Manure	--	--	*Eisenia foetida*	Horse	--	3 weeks	NA		
Vermiwash	Manure	--	--	*Eisenia foetida*	Goat	--	3 weeks	NA		
Compost	Manure	--	Okra (*Abelmoschus esculentus* L.)	--	Cow	Food waste: cow dung 1:1 (w/w)	95 days for composting	--	Growth of the plants in 100% compost was stunted and germination was also reduced at highest compost amendment	(Palanichamy et al., 2011)
Vermicompost	Manure and plant	--	Pak choi (*Brassica rapa* cv Bonsai, Chinensis group)	*Perionyx excavatus* and *Eisenia fetida*	Chicken	Layer manure with cardboard (approximately 5:1 by volume)	4-6 months vermicompost period & 3 months curing	--	Compost quality influenced nutrient extraction efficiency, microbial activity, phytohormones and total nutrient content of extracts	(Pant et al., 2012)
Compost	Manure	--	Pak choi (*Brassica rapa* cv Bonsai, Chinensis group)	--	Chicken	Layer manure and wood chips (approximately 5:1 by volume)	composted for 6 months, cured for 3 months	--		
Vermicompost	Manure and plant	--	Pak choi (*Brassica rapa* cv	*Perionyx excavatus* and *Eisenia fetida*	Chicken	Layer manure with cardboard	4-6 months vermicompost period	--		

Experiment	Input	Hydro ponic	Experimental plant	Earthworm	Animal manure	Compost source and dilution	Preparation time	Dilution used	Comments	Reference
			Bonsai, Chinensis group)			(approximately 5:1 by volume)				
Compost	Plant	--	Pak choi (*Brassica rapa* cv Bonsai, Chinensis group)	--	--	landscape waste	--	--		
Vermiwash	Manure and plant	--	Pak choi (*Brassica rapa* cv Bonsai, Chinensis group)	*Perionyx excavatus* and *Eisenia fetida*	Chicken	Layer manure with cardboard (approx. 5: 1 by volume)	4-6 months vermicompost period & 3 months curing	1: 10 (v: v)		
Compost tea	Manure	--	Pak choi (*Brassica rapa* cv Bonsai, Chinensis group)	--	Chicken	Layer manure and wood chips (approximately 5: 1 by volume)	Composted for 6 months, cured for 3 months	1: 10 (v: v)		
Vermiwash	Manure and plant	--	Pak choi (*Brassica rapa* cv Bonsai, Chinensis group)	*Perionyx excavatus* and *Eisenia fetida*	Chicken	Layer manure with cardboard (approximately 5:1 by volume)	4-6 months vermicompost period	1: 10 (v: v)		
Compost tea	Plant	--	Pak choi (*Brassica rapa* cv Bonsai, Chinensis group)	--	--	Landscape waste	--	1: 10 (v: v)		
Vermiwash	Manure and plant	--	Indian Borage (*Plectranthus ambionicus*)	*Eudrilus eugeniae*	Cow	Cow manure: soil (no ratio)	--	10% (v/v) during experiment	Increased root growth, total chlorophyll, carotenoids, and photosynthetic pigment content	(Quaik et al., 2012)
Vermiwash	Plant	--	Bringal plant (*Solanum melongena*)	*Eudrilus euginae*	--	--	--	Water: vermiwash 1:1	Highest yield with vermicompost and soil mixture	(Samadhiya et al., 2014)
Compost	Manure and plant	--	--	--	Cow (*Bos taurus*)	Soybean *Glycine* max (L.) Merr.]	4 weeks for composting and 4 weeks for curing	--	Release of P can be estimated by water extractable P concentration in compost; this may be used to estimate surface runoff and leachate P concentration	(Sharpley & Moyer, 2000)
Compost	Manure and plant	--	--	--	Chicken (*Gallus gallus domesticus*)	Mix of hay, wood chips, and corn cobs	6–7 weeks total, 21 days curing	--		
Vermiwash	Manure and plant	--	No	*Eudrillus eugeniae*	Cow	Mango leaf litter	60 days for vermicompost process	--	Use of earthworms reduced composting time (to attain similar nutrient levels after 60 days, it took up to 45 days for vermicompost)	(Sundaravadivelan et al., 2011)
Compost	Manure	--	--	--	Cow	Mango leaf litter	60 days for composting process	--		

Experiment	Input	Hydro ponic	Experimental plant	Earthworm	Animal manure	Compost source and dilution	Preparation time	Dilution used	Comments	Reference
Vermiwash	Manure and plant	--	No	*Eudrillus eugeniae*	Cow	Guava leaf litter	60 days for vermicompost process	--		
Vermiwash	Manure and plant	--	No	*Eudrillus eugeniae*	Cow	Sapota leaf litter	60 days for vermicompost process	--		
Vermiwash	Manure and plant	--	No	*Lampito mauritii*	Cow	Mango leaf litter	60 days for vermicompost process	--		
Vermiwash	Manure and plant	--	--	*Lampito mauritii*	Cow	Guava leaf litter	60 days for vermicompost process	--		
Vermiwash	Manure and plant	--	--	*Lampito mauritii*	Cow	Sapota leaf litter	60 days for vermicompost process	--		
Compost	Manure and plant	--	--	--	Mix of livestock waste	Sawdust	60 days composting period	--	Manure compost can be an alternative to chemical fertilizer to promote soil nutrient balance	(Wong et al., 1999)

2.2 Organic food production with hydroponic systems

2.2.1 Organics and hydroponics

The demand for locally and organically grown produce in controlled environment agriculture (CEA) is growing, yet little research supports or provides recommendations for appropriate substrate mixes and nutrient management in hydroponic systems (Rogers, 2017). For example, limited literature has examined the use of compost teas in hydroponics (Table 2.1). Most often, nutrients are added to the soil or sprayed on plants' leaves (Fritz et al., 2012). However, compost preparation, technical information regarding system set up, or the chemical composition of control treatments are lacking, making it difficult to compare studies (Keeling et al., 2003; Quaik et al., 2012). For instance, a variety of plants have been cultivated with compost tea that was prepared using different methodologies in Table 2.1 (Arancon et al., 2008; Mitchell, 1997; Pant et al., 2012).

The addition of municipal solid waste compost and peat with vermicompost has been considered. Vermicomposting is being used to treat industrial waste containing heavy metals that pose a constraints for the use in agriculture if left untreated (Goswami et al., 2014). One study reported that the use of vermiwash in a hydroponic system increased Indian borage root growth, carotenoid concentration, photosynthetic pigment, and chlorophyll content when compared to the control solution (Quaik et al., 2012). A similar experiment with hydroponic systems resulted in higher tomato yield when compared to chemical fertilizer (Haghighi et al., 2016). Tomato vegetative growth was enhanced with the use of vermiwash and compost teas (Allahyari et al., 2014). For cucumber, vermicompost teas resulted in higher antioxidant capacity (approximately 42%) than a control solution that contained inorganic fertilizers (Santiago-López et al., 2016).

Aquaponics create a symbiotic environment by combining hydroponic systems with traditional aquaculture. In an aquaponic system, compost tea increased oilseed rapes shoot and root dry weight by 84% and 67% respectively, when compared to an inorganic fertilizer (Keeling et al., 2003). Studies on tomato (*Lycopersicon esculentum* Mill. 'Roma VF') and marigold (*Tagetes erecta* L. 'Crackerjack') showed nutrient deficiency, and it was concluded that compost tea nutrient levels are unbalanced as they are low in nitrogen and phosphorus content, yet high in potassium, magnesium, calcium, and sodium (Jarecki et al., 2005). It possible that the high ammonium content of the solution may have reduced pH and as such, disordered nutrient uptake in these plants (Jarecki et al., 2005). To mitigate this problem, Jarecki et al. (2005) suggested that compost tea solutions be balanced to achieve yields similar to those attained with full- and half-strength Hoagland solution. More recently, the highest yield of water spinach (*Ipomoea aquatic*) was obtained when compost tea was used as a foliar spray, and this was compared to a mix of molasses and no spray (Bethe et al., 2017).

Goat, chicken, and beef manure have been used to replace inorganic fertilizers in hydroponic systems, yet the outcomes do not always converge. Manure nutrient levels are influenced by type of floor, litter, age of manure, and management practices (Parker et al., 1959). Tomatoes grown in a hydroponic system with a solution comprised of compost tea and pig manure had a 91% yield when compared to an inorganic nutrient solution; it was determined that the compost tea was low in phosphorus, calcium, and magnesium, but rich in potassium (Ryoo & Seo, 2009). A study on Swiss chard (*Beta vulgaris*) showed that chemical fertilizers are superior to manure when yield is considered (Mowa, 2015). Another study compared bat, cattle, and pig manure to commercially available chemical fertilizers when growing lettuce in a hydroponic system. The highest yield reported was with chemical fertilizer, followed by bat manure, with a fresh mass lower by 4% (Charoenpakdee, 2017). Nutrient solutions are often not analyzed, and crops vary significantly, making comparison between treatments challenging (Charoenpakdee, 2017). To address knowledge gaps with respect to the benefits of livestock manure in hydroponic systems, precise experiments comparing different manure sources at different concentrations are needed.

2.2.2 Organic certification regulations

Hydroponic systems are not considered organic in Canada, Sweden, and most European countries (NOSB, 2016). Some Scandinavian standards and the National Organic Program in the US permit hydroponics as a variant of organic farming (Jannasch, 2008; NOSB, 2016). The main argument is that organic production must be grown in the ground, which obviously does not occur in soilless methods (NOSB, 2016).

The Canadian Food Inspection Agency (CFIA) is the entity responsible for monitoring and enforcing the *Organic Products Regulations*. This document states that hydroponic and aeroponic production is prohibited, as hydroponic systems require a nutrient solution and fertilizers are prohibited at all stages of growing and harvesting of organic products (OFC, 2015). However, aquaponics, research that includes animal manure, compost teas, and soil amendments are accepted and promoted in organic agriculture, without the use or addition of inorganic fertilizers (Government of Canada, 2018; Nikmane & Klintsare, 1990). Furthermore, different compost preparation methods have been made available to the public by the Canadian Organic Growers to minimize the risk of pathogen contamination and to optimize compost nutrient composition (Government of Canada, 2018). An argument could be made in which the addition of compost, in the form of compost tea with or without manure, to a hydroponic system should be considered organic as it does not uses inorganic fertilizers and uses the same preparation processes as aquaponic production. (Canadian Organic Growers, 2001).

Canadian standards for organic crop production have their limitations. As there are no guidelines that address lighting regime. Crops grown with artificial light might also be considered organic. Further to this, there are no guidelines on soil composition and mineral content; therefore, hydroponic systems using sand to anchor roots could still meet this regulation (NOSB, 2016). Yet, there is a transition period of 36 months before the soil can be used within a greenhouse as a farmer could have used pesticide and inorganic fertilizer prior the transition to organic agriculture (OFC, 2015). These weaknesses in regulation should be addressed or clarified as food movement producing fruits and vegetables in controlled-environment agriculture gains momentum across Canada.

2.2.3 Nutrient content in compost teas

The efficacy of compost teas and vermicompost varies with feedstock, crop type, and the objectives of the grower. Furthermore, nutrient content in compost tea, vermiwash, and manure extracts is strongly dependent on the initial substrates that were used (Table 2.1) (Quaik et al., 2012). Electrical conductivity (EC) do not provide an accurate information on the concentration of individual ions and nutrients should be analyzed separately (Bamsey et al., 2012; Kim et al., 2013). Compost and vermicompost usually have higher pH and EC values than vermiwash and compost tea as the latter two are usually diluted prior application on plant (Table 2.2). Some vermicompost are not diluted and would explain their elevated pH and EC value (More et al., 2013). Methods vary from study to study and as such, comparisons between compost tea and vermicompost remain a challenge as presented in Table 2.1. To add to this problem, preparation of vermicompost is sometimes carried out by a third party, using continuous flow reactor systems. This makes methodologies difficult to replicate (Arancon et al., 2008).

Table 2.2. Average pH and EC values reported for different organic fertilizers. EC: Electrical conductivity. (Data compiled from studies listed in Table 2.1).

Treatment	**Source**	**pH ± SE** (*n* = 55)	**EC ± SE** **(mS/cm;** *n* = 43)
Compost	Manure	7.5 ± 0.2	11.7 ± 10.5
Compost	Manure and plant	7.4 ± 0.4	20.3 ± 0.0
Compost	Plant	7.2 ± 0.4	3.1 ± 0.2
Compost tea	Manure	7.0 ± 0.3	2.9 ± 1.3
Compost tea	Manure and plant	7.8 ± 0.4	3.4 ± 1.9
Compost tea	Plant	6.9 ± 0.5	1.8 ±0.2
Fertilizer	Plant	6.1 ± 0.5	1.4 ± 0.4
Vermicompost	Earthworms	7.8 ± 0.3	
Vermicompost	Manure	9.0 ± 0.4	6.4 ± 1.6
Vermicompost	Manure and plant	7.4 ± 0.3	17.3 ± 14.3
Vermiwash	Manure	7.8 ± 0.4	1.6 ± 0.4
Vermiwash	Manure and plant	7.4 ± 0.0	1.4 ± 0.1
Vermiwash	Plant	8.1 ± 0.7	

The addition of other fertilizers to the nutrient solution may provide the plant with all essential nutrients required for growth and development (Table 2.3) (El-Haddad et al., 2014). Essential nutrients are separated into macronutrients and micronutrients according to plant tissue concentration. Macronutrients include nitrogen, potassium, phosphorus, calcium, magnesium, sulfur (Table 2.3) (Taiz & Zeiger, 2002). Micronutrients include chlorine, iron, boron, manganese, sodium, zinc, copper, nickel and molybdenum (Table 2.4) (Taiz & Zeiger, 2002). Compost and vermicompost may also contain ammonia, salts and heavy metals that are detrimental to plant growth if not adequately monitored (Table 2.4) (Carballo et al., 2009). Metal and trace metal availability will be influenced by compost and vermicompost maturity (Hargreaves et al., 2008). As compost matures, the humic material increases and binds with metals decreasing their concentration (Hargreaves et al., 2008). However, it has been suggested that enhanced plant growth, germination, and flowering is not only influenced by the nutrient content of vermicompost. Rather, improved growth may be due to humic acids, plant growth hormones, and microbial diversity (Arancon et al., 2008). An initial nutrient analysis of organic fertilizer could be used to assess its potential on plant growth.

Table 2.3. Average macronutrient levels reported in different organic fertilizers (data compiled from studies listed in Table 2.1).

Treatment	**Source**	**N** **(ppm** ± SE) *n* = 48	**N-NO3** **(ppm** ± SE**)** *n* =32	**N-NH4** **(ppm** ± SE**)** *n* = 33	**P** **(ppm** ± SD**)** *n* = 61	**K** **(ppm** ± SE**)** *n* = 52	**Ca** **(ppm** ± SE**)** *n* =40	**S** **(ppm** ± SE**)** *n* = 13	**Mg** **(ppm** ± SE**)** *n* = 38
Compost	Manure	24,300.0 ± 500.0	7,627.4 ± 0.0	112.9 ± 0.0	20,100.0 ± 8,000.0	20,350.0 ± 750.0	71,300.9 ± 49,727.9		6,033.9 ± 3,019.6
Compost	Manure and plant	16,578.0 ± 3,582.1	114.0 ± 0.0	1,231.0 ± 0.0	10,479.6 ± 3,054.2	10,515.7 ± 5,162.5	27,094.3 ± 15,074.1		4,056.7 ± 1,551.2
Compost	Plant	9,360.0 ± 1,266.7	49.7 ± 21.9	26.8 ± 14.2	2,810.4 ± 1,433.0	7,080.0 ± 1,721.2	66,100.0 ± 0.0		7,400.0 ± 0
Compost tea	Manure	11,046.5 ± 10,753.5	146.1 ± 143.1	1,427.7 ± 1,421.8	1,334.7 ± 1,321.8	7,886.2 ± 7,481.6	54.2 ± 33.4	3.8 ± 3.3	39.8 ± 33.0
Compost tea	Manure and plant	6,290.1 ± 2,663.0	2,475.0 ± 929.5	135.0 ± 83.3	2,825.0 ± 2,787.5	8,399.4 ± 7,707.9	84.2 ± 81.8		62.4 ± 61.6
Compost tea	Plant	9.5 ± 0.0	3.9 ± 2.2	9.8 ± 8.1	3.7 ± 2.0	308.8 ± 44.2	144.2 ± 88.5	192.5 ± 42.5	54.4 ± 17.6
Fertilizer	Plant		95.4 ± 29.9	11.5 ± 3.7	17.8 ± 4.2	176.2 ± 35.3	86.0 ± 32.9	51.8 ± 8.2	24.5 ± 9.6
Vermicompost	Manure	29,166.7 ± 10,045.9	234.0 ± 0.0	9.1 ± 0.0	6,330.6 ± 5,272.7	21,280.0 ± 5,820.0	13,485.0 ± 12,715.0	5,900.0 ± 0.0	4,720.0 ± 4,580.0
Vermicompost	Manure and plant	18,900.0 ± 3,389.6	825.8 ± 544.7	121.0 ± 55.3	15,925.6 ± 4,674.4	20,702.3 ± 8,266.1	118,298.1 ± 58,626.7	5,710.0 ± 0.0	4,716.8 ± 1,967.6
Vermicompost	Plant	13,125.0 ± 1,125.0			5,587.5 ± 1,931.5	16,097.5 ± 6,575.3	13,510.0 ± 1,510.0	25,660.0 ± 23,940.0	3,612.5 ± 3,72.5
Vermiwash	Manure	8,100.0 ± 2,026.3	247.0 ± 0.0	7.4 ± 0.0	4,000.4 ± 1,572.3	7,400.0 ± 288.7	1,900.0 ± 600.0		
Vermiwash	Manure and plant	126.4 ± 46.6	88.8 ± 49.2	0.5 ± 0.2	3,493.8 ± 872.9	130.3 ± 57.0	36.0 ± 20.6		29.6 ± 15.6
Vermiwash	Plant	3,979.6 ± 3,760.8			4,952.4 ± 4,528.9	5,651.6 ± 4,935.3			

Table 2.4. Average micronutrient levels reported in different organic fertilizers (data compiled from studies listed in Table 2.1).

Treatment	**Source**	**Fe (ppm** ± SE**)** ***n* = 19**	**B (ppm** ± SE**)** ***n* = 9**	**Cl (ppm** ± SE**)** ***n* = 12**	**Mn (ppm** ± SE**)** ***n* = 18**	**Zn (ppm** ± SE**)** ***n* = 20**	**Cu (ppm** ± SE**)** ***n* = 20**	**Ni (ppm** ± SE**)** ***n* = 4**	**Na (ppm** ± SE**)** ***n* = 19**	**Pb (ppm** ± SE**)** ***n* = 5**	**Cd (ppm** ± SE**)** ***n* = 3**	**Cr (ppm** ± SE**)** ***n* = 4**
Compost	Manure	708.6 ± 0.0		4.4 ± 0.0	830.0 ± 0.0	657.5 ± 0.0		63.3 ± 0.0		3.0 ± 0.0	1.5 ± 0.0	20.1 ± 0.0
Compost	Manure and plant	4,757.0 ± 1212.0			311.0 ± 143.0	187.4 ± 144.0	58.6 ± 42.8	0.6 ± 0.0	7,773.3 ± 3,222.3	15.5 ± 10.6	1.2 ± 0.4	0.3 ± 0.0
Compost	Plant											
Compost tea	Manure	1.0 ± 0.9		62.7 ± 46.2	0.1 ± 0.1	37.3 ± 36.7	6.1 ± 5.9	5.5 ± 0.0	26.7 ± 19.2	6.1 ± 0.0		16.7 ± 0.0
Compost tea	Manure and plant	7.9 ± 0.0			0.2 ± 0.0	78.5 ± 78.4	95.8 ± 0.0	13.4 ± 0.0	326.0 ± 0.0	7.2 ± 0.0		39.5 ± 0.0
Compost tea	Plant	0.6 ± 0.2	0.3 ± 0.0		0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0		121.5 ± 36.5			
Fertilizer	Plant	4.8 ± 1.7	0.1 ± 0.1	317.0 ± 26.0	0.9 ± 0.5	0.1 ± 0.0			145.5 ± 48.5			
Manure	Manure											
Vermicompost	Manure	720.0 ± 0.0							13,800.0 ± 0.0			
Vermicompost	Manure and plant	1,340.8 ± 126.5	60.0 ± 0.0		57.4 ± 56.3	87.8 ± 86.1	116.9 ± 116.6		827.4 ± 822.6			
Vermicompost	Plant	14,990.0 ± 5990.0	30.0 ± 10.0		490.0 ± 190.0	265.0 ± 25.0	70.0 ± 30.0		835.0 ± 325.0			
Vermiwash	Manure											
Vermiwash	Manure and plant	2.2 ± 0.0		3.5 ± 0.0			0.4 ± 0.0		105.0 ± 0.0			
Vermiwash	Plant											

2.3 Sanitation and safety concerns

Hydroponic systems that use inorganic fertilizers have demonstrated higher sanitary quality than traditional agriculture, as there is no contact between the soil and crop plants, which minimizes presence of soil contaminants such as coliforms (Settanni et al., 2013). Yet, organic food production with hydroponic systems will require more in-depth research on the nutrient solution to minimize the growth of undesirable pathogen and the presence of chemicals potentially toxic to the consumer (Scheuerell & Mahaffee, 2002; Settanni et al., 2013).

2.3.1 Human pathogens

Manure is known to be the home of different microbiota, some of which are human pathogens (Table 2.5). Pathogen transmission and plant internalization have been investigated on plants cultivated in soil and hydroponic systems (Erickson et al., 2013). Internalization of *Escherichia coli* O157:H7 can occur through the root system and move to the leaf and stem and greater internalization event was measured when plants were grown on soil or when the roots were wounded (Erickson et al., 2013). Effects of increased level of manure production did not directly effect human health (Guan & Holley, 2003). However, the lack of proper monitoring of pathogens can cause massive disease outbreaks and infect large populations (Erickson et al., 2013; FDA, 2019). Illnesses caused by the enteropathogens *E. coli* O157: H7 and *Salmonella enterica* serovar Typhimurium cost over USD $405 million (Semenov et al., 2007). Foodborne illness outbreaks caused by *E. coli* O157:H7 occurred in 2016, 2017 and 2018 and even if the food vehicle was not confirmed, romaine lettuce remains as one of the main suspects (FDA, 2019). Pathogens found in cattle manure are easily transmitted as they have a low infective dose and high pathogenicity. Furthermore, their survival and spread depend on how manure is handled, stored, and applied (Semenov et al., 2007).

Table 2.5. Some pathogens found in livestock waste. Adapted from (Mawdsley et al., 1995).

Bacteria	Viruses	Protozoa / parasites
Acremonas spp.	Coronavirus	*Cryptosporidium parvum*
Bacillus anthracis	Enterovirus	*Ostertagia* sp.
Brucella abortus	Rotarivirus	*Giardia lamblia*
Escherichia coli		*Cooperia* sp.
Klebsiella spp.		
Leptospira spp.		
Listeria monocytogenes		
Mycobacterium tuberculosis		
Salmonella spp.		
Streptococcus spp.		
Yersinia enterocolitica		

Different pathogen transmission prevention strategies have been investigated, and proper sterilization steps are required if manure is to be used in organic hydroponic systems. Heat has been examined as one such method that could reduce the spread of these manure-borne pathogens (Semenov et al., 2007). Higher temperatures and microbe competition significantly reduced pathogen survival, whereas manure-amended autoclaved soil increased pathogen survival as there was lower presence of indigenous soil microorganisms antagonistically interacting with the pathogens. Temperatures above 30^{o}C reduced survival for only a few days (Aorigele & Yu June, 2008). Vermicompost has been used successfully as a pest control such as arthropod and parasitic nematodes (Simsek-Ersahin, 2011). Vermiwash was also successful at suppressing pathogens such as *Fusarium moniliforme* and *Phytophthora nicotianae* (Joshi et al., 2015). Furthermore, the addition of 3% $CaCN_2$ at a temperature below 30^{o}C inhibited *E. coli* growth (Aorigele & Yu June, 2008). Therefore, composting for several months, where the temperature can reach over 55^{o}C, might be a better approach to sterilizing manure than autoclaving (Semenov et al., 2007).

2.3.2 Chemicals and residues

Toxic chemicals present in the water and in manure may be uptaken by plants grown in hydroponic solutions. Industrial and human waste has been studied in hydroponic systems to monitor uptake of potentially harmful chemicals. A study conducted in 2010 investigated the uptake of human pharmaceuticals by cabbage plants (Herklotz et al., 2010). Four different pharmaceutical products were used to fortify a Hoagland solution and all plants accumulated the chemicals at different concentrations in their roots and leaves. Another experiment used textile sludge as an alternative source of nutrients (Araújo & Monteiro, 2005). Soybean and wheat were grown in solutions with increasing concentration of textile sludge. An inverse linear trend was observed, where an increase in textile sludge concentration decreased total dry matter of both plants. This response was probably caused by heavy metals present in the solution at higher concentrations (Araújo & Monteiro, 2005). Results from both studies showed that treated wastewater, also designated as reclaimed water, can still contain toxic chemicals. Additionally, presence of pharmaceuticals and personal care products can promote the development of antibiotic resistance in bacteria (Herklotz et al., 2010). Although wastewater meets regulatory requirements and therefore, can be applied as fertilizer, water might require better treatment as it can have phytotoxic effects on plants and potentially can impact human health as well (Herklotz et al., 2010).

Residues present in manure that is not adequately degraded could also represent a potential risk for humans. Tetracyclines, such as oxytetracycline (OTC), are the most commonly used veterinary antibiotics that are administered to livestock as feed supplements. These antibiotics have been found in swine manure as they are often poorly absorbed, and as high as 70-90% may be excreted, resulting manure concentrations up to 200 mg/kg (Ratasuk et al., 2012). Once in the soil, these antibiotics are considered an environmental pollutant and most notably, can transfer antibacterial resistance to soil microorganisms, generating a new bacterial strain that could present a threat to humans (Ratasuk et al., 2012). Photolysis and hydrolysis can increase degradation of OTC and be more effective than composting, as these processes require a few days before OTCs are depleted, while composting would necessitate one week at 60^{o}C (Ratasuk et al., 2012).

2.4 Waste disposal

Periodic disposal of the nutrient solution is inevitable as some nutrients accumulate in hydroponic systems to toxic levels. Sodium chloride is the most frequently accumulated salt in hydroponic systems (Savvas et al., 2004). Na and Cl adversely affect crop yield, and salts increase EC values resulting in reduced nutrient uptake (Savvas et al., 2004; Voogt & Van Os, 2010). Reuse of hydroponic solutions for salt-tolerant crops might be an alternative to reduce waste production and create a value-added crop (Rababah & Al-Shuha, 2009). If the nutrient solution is discharged, federal and municipal regulations need to be met.

2.4.1 Compost qualification in Canada

The Canadian Council of Ministers of the Environment (CCME) has qualified compost according to four criteria: foreign matter, maturity, pathogens, and trace elements (CCME, 2005). Foreign matter is defined as any organic or inorganic substance over 2 mm in dimension (length, width or thickness) that results from human intervention, including metal, glass or any synthetic polymer. Compost is mature when it does not have phytotoxic effects and when it has cured for a minimum of 21 days with a set respiration rate, carbon dioxide evolution rate, and temperature values. Guidelines also include the number of fecal coliforms and *Salmonella* permitted in compost. Trace elements are defined as chemicals present in the compost at very low concentrations.

The CCME has further separated compost in categories A and B based on the trace element concentration and sharp foreign matter content (Table 2.6). Category A may be made from municipal solid waste, pulp and paper, or manure (CCME, 2005). This category is labeled as unrestricted use and can be used in any application, including agricultural and residential lands, nurseries, and other businesses. However, trace element concentrations must meet Category A standards, and permitted amounts of Cu and Zn have been increased to allow the composting of other feedstocks, such as poultry and hog manure. Category B comprises compost that contains sharp foreign matter and higher concentrations of trace elements. This category is labeled restricted use and additional control may need to be necessary based on provincial or territorial regulations.

Sharp foreign matter in compost cannot be greater than 3 mm in dimension per 500 mL for Category A compost, and there must be less than, or equal to, three pieces of 12.5 mm in size per 500 mL in Category B composed. If compost does not meet either category, it must be used or disposed following the regulations of the province or territory.

Table 2.6. Category A and B compost categories outlined by the CCME. Adapted from (CCME, 2005).

	Category A	**Category B**	
Trace elements	**Maximum concentration within product (mg/kg dry weight)**	**Maximum concentration within product (mg/kg dry weight)**	**Maximum cumulative additions to soil (kg/ha)**
Essential or beneficial to plants or animals			
Arsenic	13	75	15
Cobalt	34	150	30
Chromium	210	--	--
Copper	400	--	--
Molybdenum	5	20	4
Nickel	62	180	36
Selenium	2	14	2.8
Zinc	700	1850	370
Other			
Cadmium	3	20	4
Mercury	0.8	5	1
Lead	150	500	100

2.5 Manure management and disposal in Quebec

In Canada, each province conforms to provincial regulations that are monitored by the ministry of environmental protection or agriculture. While the Fisheries Act under federal legislation states that there is, “a prohibition against the deposition of a deleterious substance into waters frequented by fish”, *Quebec Agricultural Operations Regulation Environment Quality Act* states the following:

“It is prohibited to discharge or deposit in the watercourse and the owners of lot must take the necessary actions to prevent livestock to enter in contact with surface or subsurface water. It is also prohibited for livestock to cross watercourses or have access to them.” (Government of Quebec, 2018a)

In addition, the following is stated with respect to livestock and livestock waste:

“Livestock waste must be at least 15 m away from any watercourse with a flow area (average width multiplied by average height) greater than 2 m^2*. The livestock must not encounter the livestock waste and the waste should not enter any water surface. Set amount of phosphorous (*P_2O_5*) must be conserved if the manure will be used for fertilization. All stored waste must be removed within 12 months or less and applied as fertilizer. The minimum area required for fertilization use can be found in Schedule I and an agrologist must have been consulted prior the fertilizer application.” (Government of Quebec, 2018a).*

Finally, the following must be considered when characterizing livestock waste: total nitrogen, calcium, magnesium, dry matter, total phosphorus, potassium, and ammonium nitrogen content, along with the carbon/nitrogen ratio. If the manure is not used, it must be destroyed, and wastewater from farms must flow into a storage facility or a sewer system (Government of Quebec, 2018a).

In Quebec, solid and liquid manure disposal are regulated under the *Quebec Agricultural Operations Regulation Environment Quality Act*, and disposal of the hydroponic solution must be processed to meet permitted standards for trace elements (Table 2.7).

Table 2.7. Maximum concentration allowed from contaminants. Adapted from (Government of Quebec, 2018b).

Contaminant	Standard (mg/L) Present in liquid leachate from solid material
Arsenic	5.0
Barium	100
Boron	500
Cadmium	0.5
Total cyanide (in liquids only)	20
Chromium	5.0
Total fluoride	150
Mercury	0.1
Nitrates + nitrites	1,000
Lead	5.0
Selenium	1.0
Uranium	2.0

2.6 Controlled environment agriculture and automation of hydroponic systems

Controlled environment agriculture allows for year-round plant production, closer deployment to the consumer and reduced transportation costs (Jensen & Collins, 1985). Hydroponic systems increase plant productions in CEA. For instance, it is possible to harvest 38 lettuce plants m^{-2} (representing 243 plants m^{-2} $year^{-1}$) in a hydroponic system, compared to only 6.5 plants m^{-2} (32.5 plants m^{-2} $year^{-1}$) in an open field production (Story et al., 2010).

Implementing “smart” systems and technologies into CEA allow for automated hydroponic systems that optimize crop growth (Story et al., 2010). This entails monitoring and controlling parameters such as air temperature, humidity, light intensity, carbon dioxide concentrations, etc. For example, an environment rich in carbon dioxide, above 600 ppm, elevates crop yield (Wong, 1979). In addition, smart sensors identify plant requirements and stresses, such as nutrient or water deficiencies, and then perform an action to remedy conditions. During cultivation, for example, if one of the measured parameters is above or below a pre-set value, the automated system will make the necessary adjustments to return the growth environment back to its set value (Patil et al., 2016).

Non-contact sensors are favored over contact sensors (also referred to as destructive measurements), to collect real-time data (Story & Kacira, 2015; Story et al., 2010).

2.6.1 Ion-selective electrodes and hydroponics

Ion-selective electrodes (ISEs) have revolutionized hydroponics. Traditionally, hydroponic systems use EC and pH electrodes to monitor nutrient concentrations in solution. Solution quality is maintained by injecting fresh nutrient solution when EC declines (Kim et al., 2013). Unfortunately, such systems do not provide accurate information on the concentration of individual ions (Kim et al., 2013). ISEs mitigate this problem and are slowly being implemented into growth systems as a key tool in assessing several factors in the environment, notably in the soil and water (De Marco et al., 2007; Forster & Keyes, 2006).

ISEs measure nutrients in closed hydroponic systems that reuse drainage solution (Hashimoto et al., 1988; Morimoto et al., 1991), and they provide affordable, non-destructive analyses that are not as time-consuming as current methods such as atomic absorption spectroscopy and inductively coupled plasma-atomic emission spectroscopy (Chen et al., 2018; De Marco et al., 2007). A recent study concluded that ISE-measured nitrate and potassium concentrations were comparable to those determined using standard laboratory instruments (Kim et al., 2013). ISEs are capable of monitoring metallic species (mercury, lead, copper and alkali metals) and anions (chloride, cyanide, fluoride and sulfide) (Forster & Keyes, 2006). The concentration of gaseous analytes may also be determined by ISEs. Some of the most commonly detected gases include carbon dioxide, nitrogen oxide, sulfur dioxide, ammonia and halides (e.g. chlorine) (Forster & Keyes, 2006). ISEs are unaffected by color and turbidity (Radu et al., 2013). While further research is needed to measure all ions of interest in freshwater, including phosphate and trace metals, high salt content appears to interfere with readings in sea water (Cuartero & Bakker, 2017; De Marco et al., 2007). Some ISEs, such as those that detect calcium and magnesium, need improvements as they have demonstrated poor sensitivity and selectivity in hydroponic solutions, and interference from other ions, such as potassium, can affect calcium measurement in mixed solutions (Kim et al., 2013).

Real-time sensors are being developed for “smart” systems that will be able to process information, compensate for baseline drift, communicate with other sensors, adapt to their environment, perform self-diagnosis and calibrate automatically (Cambra et al., 2018; Changmai et al., 2018; Forster & Keyes, 2006).

2.6.2 Ion-selective electrode components

ISE are membrane-based potentiometric devices that can measure the activity of a specific ion in solution (Kim et al., 2007). Potentiometry uses electrodes to measure electric potential difference and provide chemical information of a given solution. The potential between two electrodes is measured with the use of a high impedance voltmeter. This means that the voltmeter does not draw current from the electrochemical cell and that the potential measured is very close to equilibrium (Forster & Keyes, 2006).

An ISE has a selective membrane, an internal reference electrode and an external reference electrode (Figure 2.1) (Forster & Keyes, 2006). The selective membrane is made from glass, poly vinyl chloride (PVC) and coated wire; it allows the exchange of selected ions, either cations or anions, at the solution-membrane boundary. The ion-selective membrane produces different potentials at different ion concentrations. The ion meter measures the potential difference, which is proportional to the activity of the selected ion, between the ion-selective and the reference electrode. (Forster & Keyes, 2006). As mentioned above, the electrodes can respond selectively to specific analytes in solution or in gas phase. ISEs are known to provide highly selective and precise results (Kim et al., 2007).

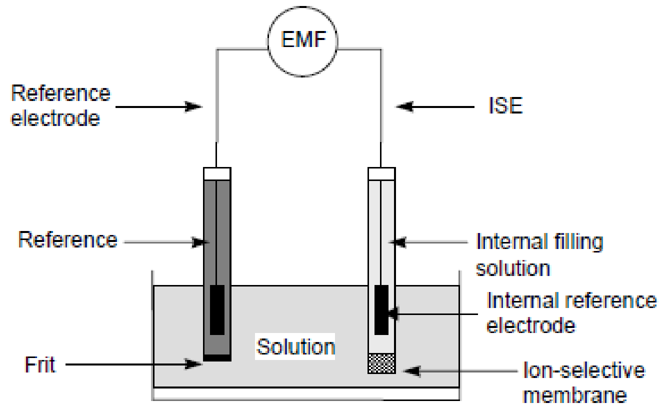

Figure 2.1. Construction of an ISE (Forster & Keyes, 2006)

The Nernst equation describes the relation between the ion activity and the measured potential difference; the electrode potential (Equation 1) (Forster & Keyes, 2006; Morf, 2012). For an ideal membrane, the slope is 59.16 mV per decade change (dec^{-1}; ex.: 0.1 to 1 mg/L) at 25°C for a univalent cation (Forster & Keyes, 2006).

Equation 1

$$E = E_0 + \left(\frac{RT}{zF}\right) ln[a_i]$$

Where:

- E is the total potential (mV) developed between the sensing and reference electrodes;
- E_0 is the standard potential of the system (mV);
- a_i is the activity of the ion in solution i;
- z is the charge of the ion (valency), with its sign, positive for cation and negative for anion;
- R is the gas constant (8.314 J mol^{-1} K^{-1});
- T is the absolute temperature (K);
- F is the Faraday constant (96,500 C^{-1} mol).

2.6.3 Ion-selective electrode types

There are four main types of ISEs: solid-membrane electrodes, liquid ion-exchange electrodes, gas-sensing membrane electrodes and solid-contact/contact-wire ISE. ISE may be combined into arrays to perform multicomponent analysis, and these arrays have multiple similar sensors, which allows for a more reliable sensing system (Forster & Keyes, 2006; Morf, 2012).

Solid membrane electrodes often have glass membranes made from lithium, aluminosilicate, or multicomponent glasses (Forster & Keyes, 2006; Morf, 2012). The main body of the electrode contains an internal reference electrode (Figure 2.2), such as Ag/AgCl. The reference electrode is filled with chloride salt. These glass membranes are primarily used for sensing monovalent cations, such as H^+, Na^+, and K^+, NH_4^+ and Ag^+ (Forster & Keyes, 2006). The glass membrane is either homogeneous, with single crystals or compressed powder pellets, or heterogeneous, where an active crystalline material is dispersed within another matrix (Forster & Keyes, 2006). Currently, no anion-selective glass membrane material has been reported.

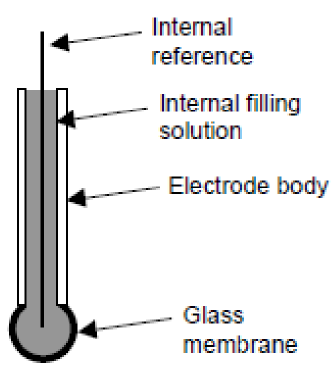

Figure 2.2. Representative image of a glass electrode (Forster & Keyes, 2006).

A liquid ion-exchange electrode contains a homogeneous, organic and water-immiscible liquid membrane (Figure 2.3). This design detects a much wider range of compounds than solid-membrane electrodes, including K^+, Ca^+ and NO_3^- ions (Forster & Keyes, 2006). However, there are more challenges with this sensor, as the liquid membrane can be unstable and may become contaminated, resulting in inaccurately read potentials (Forster & Keyes, 2006).

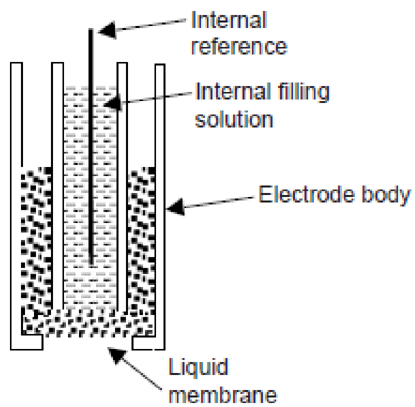

Figure 2.3. Liquid ion-exchange electrode (Forster & Keyes, 2006)

2.6.4 Ion-selective field effect transistors

Ion-selective field effect transistors (IFSET) combine ion-selective membranes with metal oxide semi-conductor field-effect transistors (MOSFET) (Bergveld, 1970; Scott, 1995). In an ISFET, the metal gate and gate oxide of the MOSFET are replaced by the sample solution with a reference electrode in contact with the solution and an insulating layer (SI_3N_4) selected on the basis of the specific analyte to be detected (Figure 2.4). The concentration of a specific ion in a sample is determined by measuring change in the gate voltage (V_{GS}) and drain current (I_D) (Figure 2.4). The gate voltage modulates the current flowing between the drain and source (V_{DS}) of the transistor so that the voltage is amplified (Artigas et al., 2001). ISFET selectivity may be varied with the addition of ion-selective membranes. ISFET have lower detection limits than ISEs, with a dynamic range of 10^{-10} M to 10^{-5} M for heavy metal ions, making them useful when it comes to measuring limits such as lead in drinking water (Chen et al., 2018).

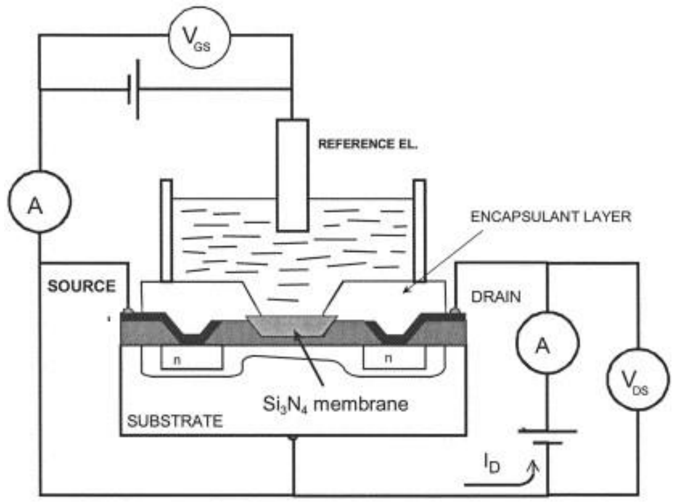

Figure 2.4. IFSET mechanism (Artigas et al., 2001)

2.6.5 ISE range and error control

The dynamic range of an ISE is the concentration range where the electrode will provide a meaningful signal (Forster & Keyes, 2006). The lower limit of detection is the lowest concentration level of analyte that can be determined as statistically different from the analyte blank (Forster & Keyes, 2006). Use of ion buffers is one method to extend the calibration ranges of ISEs. In order to have an optimal reading, there should be minimal chemical pre-treatment of the analyte, and the relationship between the signal and ion activity must be known and be specific (Forster & Keyes, 2006; Kim et al., 2007). However, calibration can be affected by the change in ionic strength of the buffer, the change or degradation of the membrane over time, the geometry and material used for ISE construction, and variability of the background composition of the analyte solution (Forster & Keyes, 2006; Kim et al., 2013).

ISEs have their own set of disadvantages and they require regular maintenance. Signal drift and reduced accuracy over time are major concerns (Kim et al., 2013). In addition, chemical interference can affect readings, but various calibration methods and the addition of other compounds, such as Ag_2SO_4, can suppress chloride interference and minimize this limitation (Kim et al., 2013). The presence of organic material in a hydroponic solution can promote growth and deposition of biofilms on the sensors, further reducing ISE reading accuracy. Interference from other ions remains a challenge for ISEs (De Marco et al., 2007), The concentration of interfering ions can significantly affect the linear calibration range and the limit of determination (Forster & Keyes, 2006).

To minimize ISE error, temperature and response time must be known before a reading is taken. In addition, use of controls, blanks and frequent calibration with known samples can help minimize error and identify possible sample contamination (Cuartero & Bakker, 2017; Forster & Keyes, 2006). Continuous exposure to the sample can create electrode drift. However, this can be prevented by minimizing contact time with the sample (De Marco et al., 2007).

2.6.6 Irrigation in hydroponic systems

Two main types of hydroponic systems exist: open and closed. In open hydroponic systems, a fresh nutrient solution is introduced for each irrigation cycle. In this system, the main objective is to ensure that the irrigation frequency and amount of water added keeps plant roots under a salinity threshold and that leaching is minimized in order to reduce waste produced by the system (Anastasiou et al., 2008). In closed hydroponic systems, the nutrient solution is re-circulated within the system. This second system's objective is to correct the nutrient solution by assessing nutrient uptake and salt build-up (Anastasiou et al., 2008).

Various factors are monitored when optimizing irrigation systems. With respect to the nutrient solution, a surplus of nutrients ranging between 20-50% of the total supply has been suggested (Schröder & Lieth, 2002). In addition, water quantity is properly determined using environmental information (temperature, humidity, and lighting), plant growth (size) and air movement (Schröder & Lieth, 2002). When assessing water that will be used for nutrient solution, ions present, dissolved materials, presence of biotic factors, particulate residues, pH and alkalinity should also be considered (Schröder & Lieth, 2002). Dissolved oxygen levels should be above 60% to prevent wilting, poor root growth or root death (Savvas et al., 2007; Schröder & Lieth, 2002). Together, collected information is used to set a baseline for water quality.

Most original patents for hydroponic systems use timers to automate water addition, while only a few record moisture in the rockwool or rooting substrate to determine crop water requirements (Chew, 1976; Lund, 1996; Rivest, 1991). Optimal irrigation schedules are still being debated, and current irrigation schedules are set by trial and error according to the grower's personal experience (Möller et al., 2007; Schröder & Lieth, 2002). Moreover, irrigation scheduling may change according to plant development and fruit setting, or external influence, such as market prices and customer preferences (Andaluz et al., 2016; Savvas et al., 2007; Schröder & Lieth, 2002). Therefore, irrigation may differ from the optimal water supply.

More novel systems use sensors and machine vision to determine crop water stress levels, and in response, activate nutrient and water pumps (Hendrawan & Murase, 2011; Kacira et al., 2002). During the transpiration process, water on the leaves' surfaces vaporizes and diffuses into the atmosphere while cooling the leaf (Kacira et al., 2002). Loss of turgor can result from the loss of water from plant cells and is a symptom of water stress in plants, but it can also be caused by salinity and low root temperature (Tazuke & Kinoshita, 2014). Changes in leaf turgidity can be determined at the canopy level and various approaches already exist to monitor leaf change, including identification of leaf movement, tracking of leaf tips and changes in leaf angle (Kacira et al., 2002).

Measurement of the top-projected canopy area (TPCA) is an alternate method for measuring water stress as older leaves are the first affected. TPCA monitoring is affected by detecting the variation among crops of fully developed leaves (Giacomelli et al., 1998). This method was previously used to determine crop growth rate (Giacomelli et al., 1998). In one study, if plants showed signs of water stress, water pumps were activated until soil moisture levels reached 45% (Kacira et al., 2002).

Infrared thermometers are used to determine canopy temperature over a large area, and this is paired with vapor pressure deficit (VPD) to determine crop water stress index (Irmak et al., 2000). Leaf temperature is inversely correlated with transpiration rate and stomatal conductance (Möller et al., 2007). Baselines for non-water stressed plants and water-stressed plants have been investigated using canopy temperature (Möller et al., 2007). In order to improve accuracy, visible imagery can be added to better separate leaves from background and canopy temperature can be isolated (Möller et al., 2007). Values collected from these sensors can be used in models to determine plant water requirements. However, determining crop water stress index is a challenging task early in the season, when partial canopy covers are present within a low plant population; if not properly considered, crop water stress can be overestimated and plants over-irrigated as a result (Irmak et al., 2000).

Collection of data by sensors to determine crop water stress allows for better adjustments to the nutrient solution and irrigation requirements of plants. Based on these data, decision support systems have been developed to improve irrigation systems and they have two functions: 1) monitor and control the irrigation system; and 2) determine when irrigation should start (Anastasiou et al., 2008). Together, sensors and models allow for the detection of crop water stress and they can automate adjustments throughout the plant's life (Schröder & Lieth, 2002).

2.7 Conclusion

The agricultural sector producing vegetables in Canada is shifting from conventional land-based agriculture to food production in CEA. With this comes a growing demand for locally and organically grown produce that has been pushed forward by stricter environmental regulations and price volatility of inorganic fertilizers. Manure, vermicompost, and compost extracts have demonstrated efficacy in optimizing crop yields and studies suggest that these organic fertilizers could at least, in part, be incorporated into hydroponic solutions, reducing the need for inorganic fertilizers. However, proper preparation methods and disposal protocols need to be documented, and best practices established if these organic extracts are to be produced on a grander scale. Ultimately, the risk of harmful pathogen transmission to the consumer must be eradicated, and waste minimized to remain sustainable.

ISE and automation can reduce manual labor, while diminishing use of unneeded fertilizers. The use of hydroponic systems in CEA can ultimately optimize productivity and reduce waste while reducing environmental degradation from fertilizer percolation into soil. This can be achieved with ISEs and smart systems that allow for continuous nutrient monitoring and adjusted responses that are more affordable and less time-consuming than traditional nutrient monitoring methods.

Connecting Text

Chapter 2 reviewed the current state of knowledge on organic fertilizers preparation methods and their use in hydroponics systems. The chapter summarized macro- and micronutrient levels in organic fertilizers based on their source. Regulations of organic waste disposals, sanitation and safety concerns were emphasized. The second section reviewed the functionality and use of ion-selective electrodes to monitor nutrient levels in hydroponic systems.

Chapter 3 presents the results of a study on the preparation of a manure extract solutions used for lettuce and kale grown in hydroponic systems. Three types of animal manure were used: chicken, cow, and turkey, to prepare the nutrient solutions at different concentrations. Ebb and flow hydroponic systems were used to complete the experiment. The highest yield was achieved with the turkey manure, while all plants died in the chicken manure, yet all treatments yield were below the control, Hoagland solution. Nutrient toxicity was assessed on plant biomass and nutrient analysis provided information on the key nutrients to monitor when preparing organic fertilizers. Pre-processing steps were suggested to reduce ammonium toxicity and promote nitrate formation. The results obtained in this study lay the foundation for future studies to optimize manure based organic fertilizers used in hydroponic systems.

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3 AERATED CHICKEN, COW, AND TURKEY MANURE EXTRACTS DIFFERENTLY AFFECT LETTUCE AND KALE YIELD IN HYDROPONICS

Abstract

Manure extracts possess great potential as alternate inorganic fertilizers. However, limited information exists on how manure influences plant growth. This study's aim was to determine the impact of aerated manure extracts on romaine lettuce *(Lactuca sativa* var. longifolia) and Russian kale (*Brassica napus* 'Red Russian'*)* in hydroponic systems. Chicken, cow, and turkey manure extract solutions (10, 25 and 50 g/L manure) were compared to a control (Hoagland) solution for lettuce and kale grown in an ebb and flow hydroponic system. The aboveground dry mass of lettuce grown in a 50 g/L turkey manure extract solution was significantly greater than that of the control. The largest aboveground wet mass for kale occurred with the control. Nutrient analyses of all manure extract solutions showed a 29% to 79% higher concentration of NH_4-N and higher total [N] than the control. Principal component analysis of the nutrient solutions identified six nutrients that should be monitored to maximize plant yield when using manure extracts in hydroponics: NO_3^-, NH_4^+, Ca, Mg, Mn, and Na. Healthy lettuce and kale plants were grown in turkey manure extract solution (50 g/L). However, reduced aboveground wet and dry mass were recorded for both plants in chicken and cow manure extract solutions compared to the control. NH_4^+ toxicity likely killed all plants grown in chicken extract (50 g/L). Apart from NH_4^+ and P, all nutrients were below suggested concentrations suggested for lettuce growth. A combined manure/mineral fertilizer may be necessary to optimize hydroponic solutions.

Keywords: *Brassica napus*, composting, hydroponics, *Lactuca sativa*, manure

3.1 Introduction

Hydroponic plant production is a soilless agricultural practice that comprises a nutrient solution and an artificial rooting substrate to support plant growth (Kumar & Cho, 2014). Hydroponic systems are mostly employed in greenhouses, where the environment can be controlled and plant production can occur year round (Lee & Lee, 2015). Under these conditions, pest control problems, pesticide use, and weed growth are considerably reduced (Surendran et al., 2016; Tomasi et al., 2015). In addition, hydroponic systems often use less water and have higher water efficiency, as nutrient solutions are not lost to ground infiltration. Nutrient solutions are reused in a closed system and can also be treated on site before being discarded (Surendran et al., 2016). As such, the productivity per unit area of a hydroponic system is higher than that of field-grown agriculture, and growing crops in controlled environments allows for the cultivation of plants with higher and more uniform nutritional values (Surendran et al., 2016; Suvo et al., 2017).

As soil is not required in hydroponic systems, conventional issues such as inadequate soil fertility, soil erosion, and compaction are absent. The use of organic fertilizers in hydroponics systems, however, remains a challenge as achieving plant yields comparable to those grown with inorganic fertilizers is more difficult, balancing nutrient solutions is problematic, and the pH and electrical conductivity (EC) of solutions fluctuate considerably (Burnett et al., 2016). In recent years, alternate hydroponic substrates derived from solid municipal waste have been considered (Haghighi et al., 2016). In organic farming, “compost teas” have been investigated as a replacement or supplemental fertilizer (Carballo et al., 2009). Compost teas are watery extracts prepared with compost or compost mixed with manure, to cultivate beneficial microorganisms. Nutrients found in compost teas originate from the compost, or they may be added from external sources (Hargreaves et al., 2008; Ingham & Millner, 2006). They are sprayed on agricultural soils and plants, reportedly stimulating root and vegetative growth, while increasing soil microbial activity (Haghighi et al., 2016; Haller et al., 2016; Pane et al., 2016). The remedial characteristics of compost teas have garnered increasing interest as they promote antifungal activity and reduce the severity of several plant pathogen diseases including early blight (*Alternaria solani*) and grey mould (*Botrytis cinereal*) (Nartey et al., 2017; On et al., 2015; Welke, 2005).

Compost teas may be aerated or non-aerated. Aerated compost teas are compost extracts that are brewed with biological and non-biological materials such as molasses, kelp, rock dust, and humic-fulvic acids to maximize microbial growth (Ingham, 2000). They are brewed in an aerobic environment for an 18 h to 36 h period that may be further extended (Scheuerell & Mahaffee, 2002). Non-aerated compost teas are produced by steeping compost in water for several days to weeks (Ingram & Millner, 2007). For both aerated and non-aerated compost teas, fermentation parameters can influence the composition and population of microbial species (Scheuerell & Mahaffee, 2002).

In Canada, lettuce and kale were amongst the top five vegetable imports in 2016, representing $447 million and $374 million in annual sales, respectively (Agriculture and Agri-Food Canada, 2017). During the same period, lettuce grown in Canada generated $112 million in revenue. To meet the growing demands of the Canadian vegetable market, new methods must be developed to increase production and decrease cost. Animal manure has been suggested as a nutrient source as it is inexpensive and readily available (Agriculture and Agri-Food Canada, 2017).

The benefits of using compost teas prepared with sheep, beef, chicken, and cow manure, municipal solid waste, or vermicompost, in soil-based crop production systems have been extensively reported (AboSedera et al., 2015; Abul-Soud et al., 2016; Duffy et al., 2004; Fritz et al., 2012; Hargreaves et al., 2008; Jack & Nelson, 2010; Radin & Warman, 2011; Zhai, 2009). Animal manure has high cation exchange capacity and contains all the required plant nutrients that can prevent micronutrient deficiency in crops (Nelson, 2011). Nevertheless, the use of animal manure requires monitoring of ammonium, sodium, and iron levels, as well as overall nutrient solution content to control toxicity (Garraway, 1982).

Investigations examining the effectiveness of animal manure on plant growth in hydroponic systems to date have reported differing results (Garland et al., 1999; Garraway, 1982; Ingham, 2002; Leudtke, 2010). For instance, it has been reported that chicken manure-based vermicompost tea containing N-indole-3-acetic acid, cytokine, gibberellins, and humic acid promotes tomato and lettuce seed germination and seedling growth (Arancon et al., 2012). Contrarily, another study concluded that use of the nutrient film technique with chicken manure was inferior to a control hydroponic solution with respect to lettuce plant biomass yield (El-Shinawy et al., 1999). Together, previous study caveats include a lack of uniformity, insufficient monitoring, and an absence of appropriate controls when applying manure extract preparations to seeds and plants in hydroponics.

The objective of this study was to determine the impact of aerated manure-based extracts on lettuce and kale growth and yield in hydroponic systems using three manure sources (chicken, cow, and turkey) and different concentrations (10 g/L, 25 g/L, and 50 g/L) as described previously (Luedtke 2010 and El-Shinawy et al. 1997). Data support the continued monitoring of several key nutrients in manure extracts to avoid toxicity, while demonstrating that different manure sources and manure extract solution concentrations differentially impact plant yield when grown hydroponically. Further to this, these data fill critical knowledge gaps when considering the future use of manure-derived or supplemented manure-derived solutions in hydroponic systems.

3.2 Materials and methods

3.2.1 Germination and plant growth

Red Russian kale (*Brassica napus* var. 'Red Russian') (S8422-001, Richters, Goodwood, ON, Canada) and romaine lettuce (*Lactuca sativa* var. longifolia) (Ridgeline Pelleted MT0 OG, Johnny's, Winslow ME, US) seeds were germinated in pre-washed rockwool rooting medium cubes with dH_2O (Grodan, Roermond, Netherlands). For each plant species, 12 cubes were seeded (two seeds per cube) in a growing tray and transferred into a growth chamber (CMP4030, Conviron, Inc., Winnipeg, MB, Canada) for two weeks with a constant temperature of 25^oC, 50% relative humidity, and a 16 h photoperiod. Deionized water was added when necessary to maintain water levels. One week after germination, seedlings were thinned to one seedling per rockwool cube and grown for one more week in the growth chamber. A total of 12 kale and 12 lettuce seedlings were then placed in 10-cm rockwool cubes and transferred to an ebb and flow hydroponic system in the greenhouse. The seedlings were equally distributed and randomly placed within six hydroponic beds to minimize edge effect (Figure 3.1).

All plants were harvested and dried 28 d after being transferred to the greenhouse (42 d total). At harvest, the following characteristics were measured: aboveground wet mass and the number of mature leaves (≥ 2 cm in diameter). Plant samples were oven-dried at 40°C for 5 d and dry mass was measured with a scale (APX-150, Denver Instruments, Bohemia, NY, US). Dried samples were ground (CBG100SC, Black & Decker, Towson, MD, US) and stored in plastic containers until plant tissue nutrient analysis were performed.

3.2.2 Preparation of manure-derived hydroponic nutrient solutions

Cow and chicken manure were sourced from McGill University's Macdonald Campus Farm in Sainte-Anne-de-Bellevue, QC, Canada. Turkey manure was sourced from Aviculture KDEM, Inc. (Saint-Gabriel-de-Valcartier, QC, Canada), where it was left outside and exposed to the external environment for 1–6 months prior to use. For each manure type, three aerated manure extract solutions were prepared with different concentrations as described previously (Luedtke 2010 and El-Shinawy et al. 1997). In brief, 20 g, 50 g and 100 g manure were separately diluted in 30 L tap water. Solutions were aerated with an air pump for 48 h then filtered through a 1-mm sieve to remove large fragments. Next, the solutions were diluted with tap water to reach a final volume of 60 L with concentrations of 10 g/L, 25 g/L and 50 g/L. Solutions were replaced every 14 days with freshly prepared solutions.

Figure 3.1. Placement of 24 rockwool cubes with kale and lettuce seedlings after transfer to an ebb and flow hydroponic system

Identical ebb and flow (recirculating) hydroponic systems were set up for each experimental solution, including the control Hoagland solution. Each bed was flooded for 15 min every hour, resulting in a water depth of no more than 5 cm per bed. One bed contained modified full-strength Hoagland solution (Hoagland & Arnon, 1950) as a control. The other beds contained the chicken, cow, and turkey manure extract solutions at three different concentrations (10 g/L, 25 g/L and 50 g/L). Three replicas per plant (lettuce and kale) were grown for each manure source and each concentration.

3.2.3 EC and pH of manure-derived hydroponic nutrient solutions

EC and pH of each manure extract solution were measured every 2 d during the growth period in the greenhouse with a handheld EC meter (Hanna EC/TDS meter, Woonsocket, RI, US) and an AB15 pH meter (Fisher Scientific, Waltham, MA, US).

3.2.4 Manure-derived hydroponic nutrient solution analysis

Three 50 mL samples of each manure extract solution were taken on a weekly basis. Samples were filtered with a Whatman qualitative filter paper No 1 (GE Healthcare Life Sciences, United Kingdom), and stored in a freezer at -78°C prior to analysis.

The ammonium concentration of each manure extract solution was determined using the Quickchem® method for ammonium, as per the manufacturer's instructions (Lachat Instruments, Model Quick Chem 8500, Milwaukee, WI, US). Briefly, equal amounts of buffer solution and sample was mixed. The buffer solution contained phosphate, salicylate, and hypochlorite. The chemical reaction was amplified by heating the solution to 60°C. Ammonium was quantified by measuring the resulting green color at 660 nm with a flow injection autoanalyzer, according to the manufacturer's instructions (Lachat Instruments).

The Quickchem method (Lachat Instruments) was also used to determine the nitrate concentration of each solution. A total of 5 mL of each sample was passed through a copperized cadmium column (Cu-Cd column) that reduced nitrates into nitrites (Otsuki 1978). When combined with a sulfanilamide and phosphoric acid reagent, nitrites turned magenta and this colour was quantified at 520 nm with a flow injection analyser, according to the manufacturer's instructions ().

To determine the total nitrogen content in leaf tissues, 0.16 g of ground dry sample was digested with an equivalent volume of extraction solution in an autoclave for 30 min at 120°C. Next, an alkaline persulfate solution was added and nitrogen was quantified as described previously (Ebina et al., 1983). Total P was measured using the QuickChem® method for phosphorus according to the manufacturer's instructions (Lachat Instruments). Following digestion with ammonium molybdate in an ascorbic-reducing solution, the concentration of phosphorus was determined with a flow injection analyzer at 880 nm as described previously (Cabrera & Beare, 1993) and according to the manufacturer's instructions (Lachat Instruments).

3.2.5 Elemental analysis

Concentrations of several elements (K, Mg, Na, Ca, Mn, Fe, Cu, and Zn) were determined as described previously (Zarcinas et al., 1987). Briefly, each manure extract solution was diluted 20-fold with ddH_2O prior to analysis. For leaf tissue analysis, 0.16 g dried leaf tissue from each plant was incubated overnight with 10 mL nitric acid. The following day, samples were heated for 4 h at 120°C, then transferred and diluted with ddH_2O in a 50 mL Falcon tube. The samples were analyzed with the Varian 820-MS ICP Mass Spectrometer (Analytik Jena, Jena, Germany). Analysis of pathogens present in the manure extracts were not completed in this study.

3.2.6 Statistical analysis

Principal component analysis (PCA) with JMP version 13.2.1 (SAS, Cary, NC, USA) software was used to determine the effect of nutrients in the manure extract solutions on plant wet and dry mass. Analysis of variance (ANOVA) was conducted to determine differences among manure extract solutions. Post-hoc comparisons were accomplished using Tukey's honest significance test (HSD). A significance level of $\alpha < 0.05$ was employed for all statistical tests. Statistical analyses were run separately for kale and lettuce. Mean values were calculated for nutrients and biomass. JMP version 13.2.1 software was used to construct all graphs.

3.3 Results and discussion

3.3.1 Plant yield

Lettuce grown in turkey manure extract (50 g/L) solution yielded the greatest aboveground wet mass average (19.1 ± 2.7 g; 95% CI [13.6, 24.6]), followed by lettuce grown in the control (Hoagland) solution (17.5 ± 0.7 g; 95% CI [16.0, 19.0]) (Figure 3.2). Kale grown in Hoagland solution yielded the greatest aboveground wet mass average (11.9 ± 0.5 g; 95% CI [10.8, 13.0]), followed by kale grown in the turkey manure extract (50 g/L) solution (6.1 ± 1.0 g; 95% CI [4.1, 8.1]). For lettuce, the highest aboveground dry mass was recorded when grown in turkey manure extract (50 g/L) solution (1.4 ± 0.2 g; 95% CI [1.0, 1.8]), followed by lettuce grown in Hoagland solution (0.9 ± 0.0 g; 95% CI [0.8, 1.0]; $\alpha < 0.05$). Similarly for kale, the highest aboveground dry mass was measured with the turkey manure extract (50 g/L) solution s (0.8 ± 0.0 g) followed by kale grown in Hoagland solution (0.8 ± 0.1 g; 95% CI [0.7, 0.9]; Figure 3.3).

When examining how chicken manure affected plant yield, data indicated that the higher the chicken manure concentration, the more detrimental effect it had on plants. More specifically, all plants died when grown in the chicken manure extract (50 g/L) solution. For cow manure extract solutions, increases in aboveground wet mass were observed for kale (1.1 ± 0.0 g; 95% CI [1.0, 1.2] to 3.4 ± 0.4 g; 95% CI [2.5, 4.2]) and lettuce (3.1 ± 0.1 g; 95% CI [2.9, 3.4] to 6.6 ± 1.0 g; 95% CI [4.7, 8.6]) as the manure concentration increased from 10 g/L to 50 g/L. For turkey manure extract solutions, an increase in yield was observed as the manure concentration increased from 10 g/L to 50 g/L; however, the lowest plant yield was recorded for plants grown in turkey manure extract (25 g/L) solution. It is possible that the microbial and nutrient composition of the turkey manure was affected when it was exposed to the outside environment for 1–6 months prior to collection (Shinohara et al. 2011). Importantly, plants grown in the turkey manure extract (50 g/L) solution exhibited a significantly higher yield with greater NO_3, Mg, and Mn content than plants grown in either chicken or cow manure extract solutions ($\alpha < 0.05$) (Table 3.1). Further increases in manure concentration, above 50 g/L, should be considered for turkey and cow manure to identify a growth threshold for both plant species, as was observed for plants grown in chicken manure-derived solutions at 50 g/L.

To determine the effect of different manure extract solutions on leaf production, total mean numbers of leaves for both lettuce and kale in all nutrient solutions were compared (Figure 3.4). The highest mean number of leaves was recorded for lettuce grown in turkey manure extract (50 g/L) solution (10.5 ± 0.4 leaves), followed by Hoagland solution (7.3 ± 0.1 leaves). For kale, the highest mean number of leaves was counted for plants grown in Hoagland solution (7.3 ± 0.1 leaves), followed by the turkey manure extract (50 g/L) solution (6.5 ± 0.2 leaves).

It has been suggested that the optimal pH for hydroponic solutions ranges between 5.6 and 6.5 (Adams, 1997). In comparison, the manure extract solutions used in this study ranged between 7.8 and 8.2, while EC values ranged between 0.55 and 1.62 mS/cm (HI 98312, Hanna Instruments, Woonsocket, Rhode Island, US) (Table 3.2 and Figure 3.5). It should further be noted that the pH of the control Hoagland solution in this study was approximately 7.5, and the EC was 2.1 mS/cm over for the duration of the experiment.

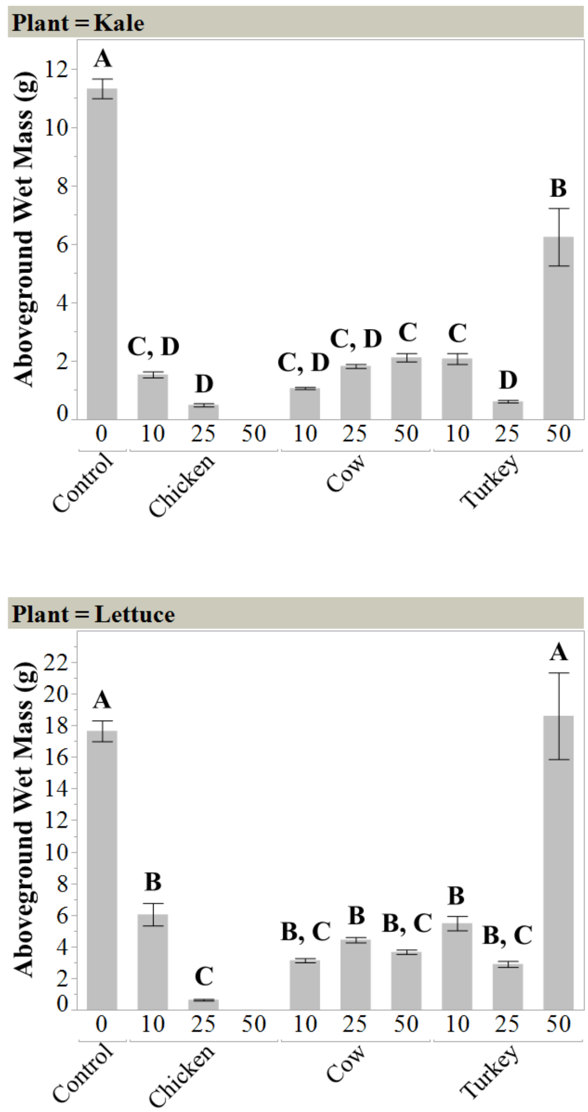

Figure 3.2. Mean aboveground wet mass values for manure extract nutrient solutions (± standard error), using Hoagland solution as the control. Letters above the columns show HSD statistical significance ($\alpha < 0.05$). Mean values for plants grown in the 50 g/L chicken manure extract solution were not included in this analysis, as none were viable at this concentration.

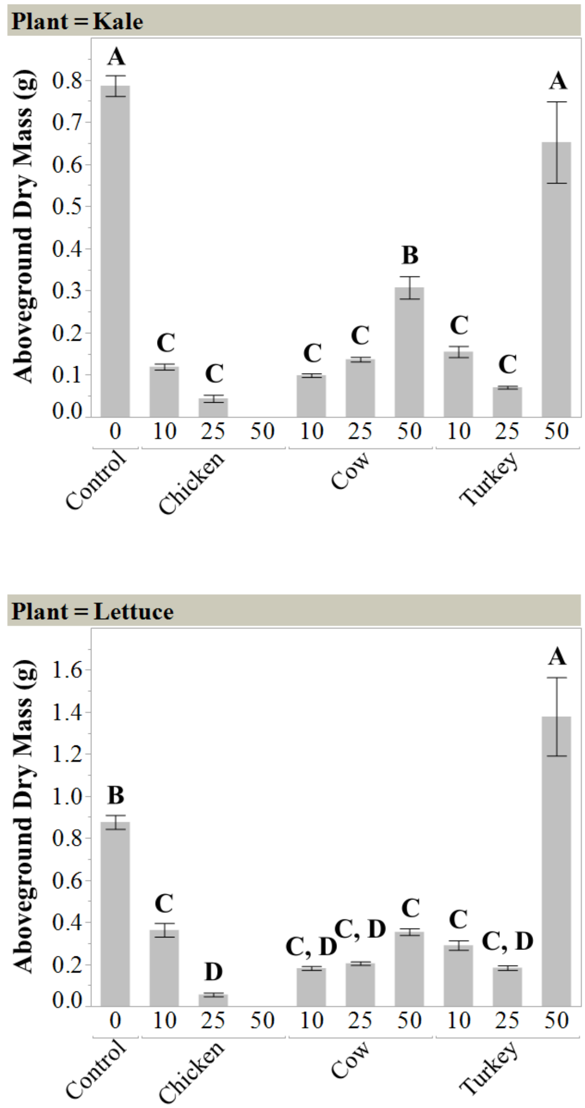

Figure 3.3. Mean aboveground dry mass for kale and lettuce grown in different manure extract solutions (± standard error), using Hoagland solution as the control. Letters above the columns indicate HSD statistical significance ($\alpha < 0.05$). Mean values for plants grown in the 50 g/L chicken manure extract solution were not included in this analysis as none were viable at this concentration.

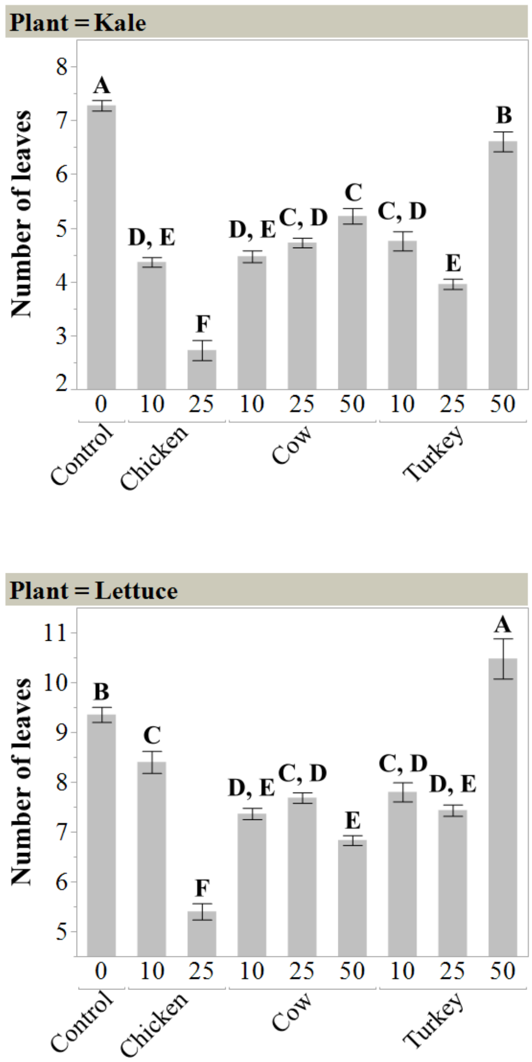

Figure 3.4 Mean total number of leaves for kale and lettuce grown in different manure extract solutions (± standard error), using Hoagland solution as the control. Letters above the columns indicate HSD statistical significance ($\alpha < 0.05$).

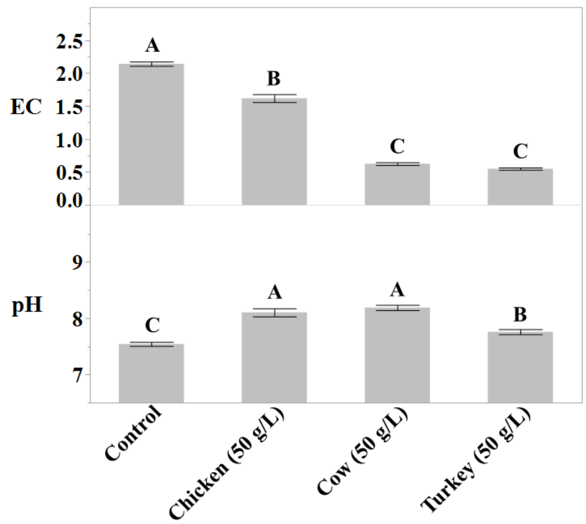

Figure 3.5 Mean EC and pH values for each manure extract solution (± standard error), using Hoagland solution as the control. Letters above the columns indicate HSD statistical significance ($\alpha < 0.05$).

3.3.2 Nutrient variation between chicken, cow, and turkey manure extract solutions

PCA identified six key nutrients to monitor in the aerated manure extract solutions that could predict plant yield. For both lettuce and kale plants, there were two clusters: 1) NO_3, Ca, Mg, and Mn; and 2) NH_4 and Na, as identified in Figure 3.6 A) and B). The first four nutrients (NO_3, Ca, Mg, and Mn) were positively correlated with aboveground wet and dry mass, while a negative correlation was identified for NH_4 and Na.

The control Hoagland solution contained the highest NO_3 concentration, followed by the turkey manure extract (50 g/L) solution, with 676.9 ± 93.9 mg/L and 52.0 ± 24.5 mg/L respectively (Table 3.1). For chicken manure extract solutions, the NH_4 concentration increased as manure concentrations increased, with a maximum of 366.6 ± 28.9 mg/L at 50 g/L chicken manure. For all cow manure extract solutions, NH_4 concentrations remained low and stable at approximately

16 mg/L. For turkey manure extract solutions, NH_4 concentrations fluctuated, peaking at 164.2 ± 14.8 mg/L in the 25 g/L solution.

The presence of sodium in the manure extract solutions was significantly higher (> 40 mg/L), in the chicken manure extract (50 g/L) and turkey manure (25 g/L) extract solutions. The lowest concentration was measured in the control Hoagland solution, at 9.4 ± 0.7 mg/L. Calcium and magnesium exhibited similar trends, where an increase in manure concentration increased both nutrients' concentrations (Table 3.1). The highest concentration of calcium was recorded in the control Hoagland solution at 159.0 ± 13.6 mg/L. The highest concentration of magnesium was measured in the cow manure extract (50 g/L) solution at 45.4 ± 3.0 mg/L, but it was not significantly different from the control (39.1 ± 2.9 mg/L). Manganese concentrations fluctuated between and within different manure extract solutions. The highest concentrations of manganese were measured in the 50 g/L turkey manure extract solution at 380.3 ± 65.1 μg/L, and the control Hoagland solution at 399.7 ± 15.9 μg/L.

Table 3.1 Nutrient variation in the manure extract solutions.

		NO3		NH4		Na		Ca		Mg		Mn	
Treatment	Conc.	mg/L	S.E.	mg/L	S.E.	mg/L	S.E.	mg/L	S.E.	mg/L	S.E.	ug/L	S.E.
Control	60 L	676.9 ^{A}	93.9	0.7 ^{D}	0.1	9.4 ^{E}	0.7	159.0 ^{A}	13.6	39.1 $^{A, B}$	2.9	399.7 ^{A}	15.9
Chicken	10 g/L	0.5 ^{B}	0.4	100.1 $^{C, D}$	14.5	12.1 ^{E}	0.7	16.7 $^{D, E}$	1.3	5.9 $^{E, F}$	0.9	112.0 ^{B}	27.6
	25 g/L	2.0 ^{B}	1.1	221.9 ^{B}	11.0	30.6 $^{B, C, D}$	6.2	21.0 $^{D, E}$	0.4	13.4 $^{D, E, F}$	1.6	217.5 $^{A, B}$	68.6
	50 g/L	2.7 ^{B}	0.9	366.6 ^{A}	28.9	50.2 ^{A}	2.4	44.0 ^{C}	0.6	20.6 $^{C, D}$	1.6	91.9 ^{B}	19.9
Cow	10 g/L	1.5 ^{B}	0.8	17.9 ^{D}	0.9	12.5 $^{D, E}$	0.5	34.0 $^{C, D}$	1.8	10.6 $^{D, E, F}$	0.9	105.5 ^{B}	12.2
	25 g/L	3.6 ^{B}	2.7	16.6 ^{D}	2.1	14.4 $^{C, D, E}$	1.2	50.7 $^{B, C}$	4.4	20.6 $^{C, D, E}$	0.7	196.5 $^{A, B}$	42.1
	50 g/L	15.7 ^{B}	5.0	15.4 ^{D}	3.7	29.6 $^{B, C}$	4.4	62.4 ^{B}	1.8	45.4 ^{A}	3.0	176.2 ^{B}	15.7
Turkey	10 g/L	8.0 ^{B}	4.0	37.1 ^{D}	12.4	19.3 $^{C, D, E}$	0.8	10.4 ^{E}	1.3	4.0 ^{F}	0.4	41.0 ^{B}	8.4
	25 g/L	1.9 ^{B}	0.6	164.2 $^{B, C}$	14.8	42.2 $^{A, B}$	4.0	13.8 ^{E}	1.6	3.5 ^{F}	0.2	95.7 ^{B}	7.2
	50 g/L	52.0 ^{B}	24.5	45.4 ^{D}	14.1	29.3 $^{B, C}$	1.8	23.2 $^{D, E}$	1.5	29.1 $^{B, C}$	3.7	380.3 ^{A}	65.1

Mean nutrient concentrations in the chicken, cow, and turkey manure extract solutions (± standard error [SE]) for nitrate (NO_3), ammonium (NH_4), sodium (Na), calcium (Ca), magnesium (Mg), and manganese (Mn). These nutrients were selected based on PCA results (Figure 6). Full-strength Hoagland solution acted as the control. Different letters next to mean values indicate statistical significance ($\alpha < 0.05$).

Table 3.2 Target nutrient concentrations in solutions for lettuce. Modified from (Baudoin et al., 2013).

Desired characteristics	Target concentration in the root environment (mg/L)
EC	2.60
pH	5.6-6.5
Ca	292.6
Mg	38.9
NH4	<10.8
NO3	1116.0
Mn	54.9
Na	<50; For sodium, this is the concentration in water used to prepare the nutrient solution

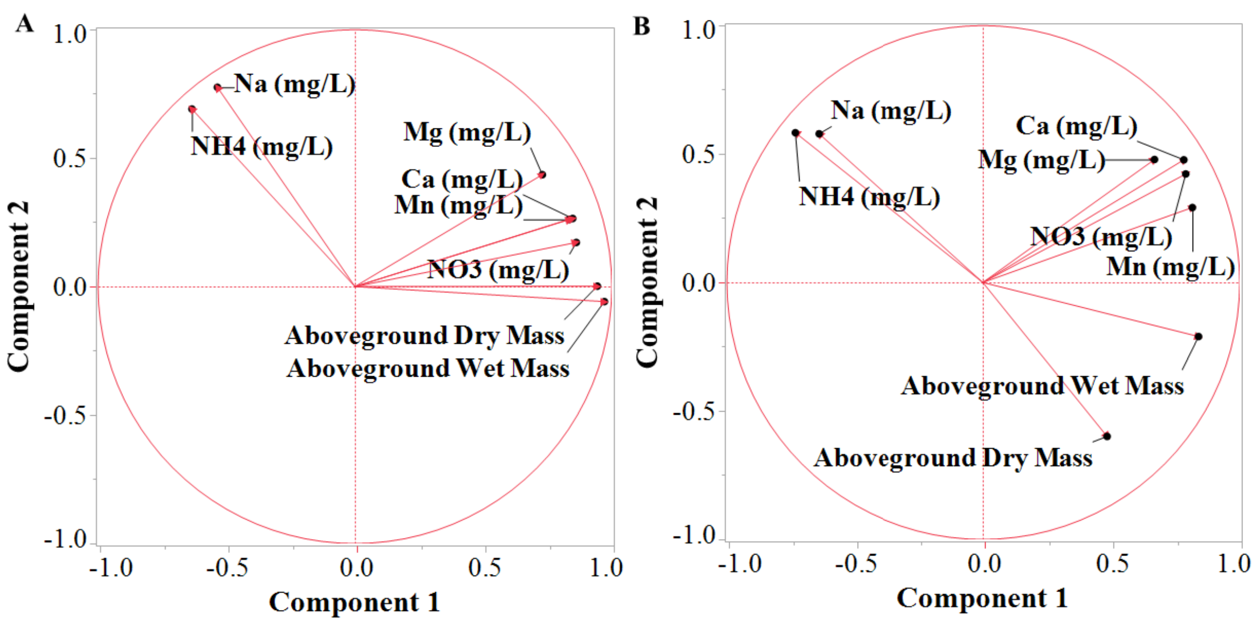

Figure 3.6. Principal component analysis of six main nutrients identified for A) kale and B) lettuce yield. For both plants, 24 nutrients were originally measured in each manure extract solution. For kale, the first principle component was 65.1% and it correlated with an increase in aboveground wet and dry mass for Ca, Mn, Mg, and NO_3. Component 2 was 18.0% and Na correlated with an increase in NH_4. For lettuce, the first principal component was 53% and it correlated with four variables. Component 1 increased with increasing aboveground wet mass for Ca, Mn, Mg, and

NO_3. Component 2 was 22.5%. It increased with only two values, Na and NH_4, and decreased with aboveground dry mass.

3.3.3 Nutrient variation in manure extract solutions and plant responses

Nitrogen

Manure extract solutions containing the highest nitrate concentrations, including the control solution and the turkey manure extract (50 g/L) solution, resulted in the greatest biomass. Nitrate is the preferential form for nitrogen uptake by plants, and the relationship between nitrate uptake and plant growth in hydroponics has been previously reported (Shinohara et al., 2011). Only turkey and cow manure extract solutions containing 50 g/L manure had detectable amounts of nitrate, yet plant yields were not significantly different from the other manure extract solutions, as the mean values that were calculated varied greatly (up to 52.0 ± 24.5 mg/L) (Table 3.1). Nitrate concentrations in the three different chicken manure extract solutions (10 g/L, 25 g/L, and 50 g/L manure) were negligible (< 5.0 ppm). In the control solution, calcium nitrate was used as the source, and this explains the higher concentration of NO_3 in this solution. As for ammonium, a linear response was observed in yield for plants grown with chicken manure extract solutions as manure concentrations increased (Table 3.1). This correlation for ammonium was not present in plants grown with cow manure extract solutions, whereby ammonium concentrations remained stable (standard error ± 4 mg/L).

Ammonium is toxic to plants at higher concentrations (Britto et al., 2001; Savvas et al., 2006; Sonneveld & Voogt, 2009). Plants that are susceptible to ammonium toxicity lack the ability to exclude ammonium through the plasma-membrane influx system and therefore, accumulate excessive amounts in the cytosol (Goyal & Huffaker, 1984). Recent studies on barley grown with a high external ammonium concentration, showed a 41% increase in total root respiration imposed by the plasma membrane on the plant root system, and a decline in plant growth (Goyal & Huffaker, 1984). This increase in energy expenditure, coupled with a lack of calcium, potassium, and magnesium in the nutrient solution, may have contributed to the apparent toxicity displayed by the lettuce and kale plants in this study. Additionally, Hoque et al. (2007) previously observed that when a nutrient solution with a nitrogen concentration of 50 mg/L is used, a reduction in plant yield is observed when the nitrogen source is ammonium; however, this reduced yield is not observed when the nitrogen source is in the form of nitrate (Hoque et al., 2007).

Recent studies have set different limits for ammonium concentrations in hydroponics. For the manure extract solutions prepared in this study, nitrogen in the form of ammonium comprised 29%–79% of total N content, and this could partly explain the lower yield recorded for plants grown in the manure extract solutions when compared to the control. That stated, the presence of ammonium at a low concentration may favor plant growth. It has been demonstrated that a supply of 4.7% NH_4-N can stimulate lettuce growth and enhance phosphorus uptake (Savvas et al., 2006), while Adams (1999) recommends an NH_4-N concentration between 10%–20%. In most commonly used nutrient solutions for greenhouses, however, 40% of total nitrogen is in an ammonia-plus-urea form, and this is considered ideal for plant growth (Adams, 1997; Nelson, 2011). It is possible that the high levels of ammonium measured in the turkey manure (25 g/L) solution were due to sampling from a fresher point in the manure pile.

The addition of microorganisms can promote ammonification and nitrification in hydroponic systems (Shinohara et al., 2011). When using fish-based fertilizer, for example, the addition of 5 g/L bark compost as a source of microorganisms is needed to mineralize organic fertilizer into nitrate (Shinohara et al., 2011). In this study, low manure concentrations and a lack of additional fertilizer might not have favored the development of nitrifying bacteria. However, pre-composting manure and forced aeration might address this problem. In this study, turkey manure that was exposed to the outside environment for one to six months contained a lower concentration of ammonium (between 37.1 mg/L and 164.2 mg/L) when compared to fresh chicken manure extract solutions (between 100.1 mg/L and 366.6 mg/L). It is plausible that leaching may have resulted from exposure to the outside environment, which enhanced microbial activity that could have promoted ammonification and nitrification. This might explain the higher nitrate concentration present in turkey manure extract solutions when compared to chicken manure extract solutions (for turkey manure extract solutions, the nitrate concentration ranged between 8.0 mg/L and 52.0 mg/L; for chicken, it ranged between 0.5 mg/L and 2.7 mg/L). Tiquia and Tam (1998) used forced aeration on pig litter sludge for 80 days, noting a sharp decrease in NH_4-N and an increase in NO_3-N and NO_2-N within the first 20 days of composting (Tiquia & Tam, 1998). Cáceres et al. (2006)

found comparable results for both NH_4-N and NO_3-N in composted cattle manure, however the experiment lasted 180 days (Cáceres et al., 2006). The addition of fertilizer and forced aeration could improve the population of microorganisms present in the manure extract, enhancing nitrate while decreasing ammonium and creating a more suitable environment for plant growth.

Within the different manure extract solutions, the potassium: nitrogen ratio was always above 4, even for the control Hoagland solution. Elevated levels of potassium can reduce calcium and manganese uptake, and most vegetative plants require a potassium: nitrogen ratio of 1.2, while fruit-bearing plants can require a higher ratio of 1.5:1 (Adams, 1997). For lettuce and tomato plants, ideal potassium: nitrogen ratios are 1.7:1 and 2.5:1, respectively. For the manure extract solutions analyzed in this study, total dissolved nitrogen was often below 200 mg/L, and it was often present as ammonium. In hydroponically grown plants, the recommended level of nitrogen is approximately 200 mg/L, and for phosphorus it is approximately 30 mg/L. However, a nitrogen source between 125–200 mg/L and a phosphorus concentration between 10–30 mg/L is acceptable (Adams, 1997). It is further recommended that potassium and magnesium concentrations be maintained below 400 and 80 mg/L, respectively, as higher concentrations can prevent calcium uptake (Adams, 1997) . For the manure extract solutions in this study, potassium concentrations ranged between 27.2 mg/L and 228.2 mg/L, while magnesium concentrations were between 3.5 mg/L and 29.1 mg/L. Since most nutrients were lower than the ones deemed optimal for plant growth, adjusting the manure extract solutions could minimize plant nutrient deficiency and prevent some plant diseases.

Sodium

It is currently recommended that salt concentrations be kept below 1,200 mg/L for optimal plant growth (Cáceres et al., 2006; Nowak & Rudnicki, 1990; Urban & Urban, 2010). In this experiment, the highest sodium levels were present in the chicken manure extract (50 g/L) solution at 50.2 mg/L, and the sodium concentration peaked at 42.2 mg/L in the turkey manure extract (25 g/L). These data show that the lettuce and kale plants likely did not suffer from salt toxicity.

High salt concentrations can improve cucumber coloration but also causes retarded plant growth at high salt concentration (Janse, 1988, 1989). High salt concentration can also limit yellow coloring and leave russeting for tomatoes, while maintaining lettuce leaf firmness (Urban & Urban, 2010). In addition, some degree of salinity can increase lettuce yield and turgor, but results vary between cultivars (Bartha et al., 2015).

Nutrient solution analyses demonstrated that sodium and ammonium concentrations were similar, and increased manure concentrations correlated with increased sodium and ammonium levels (Figure 3.6). Based on leaf tissue analysis, it was determined that sodium concentrations decreased in lettuce and kale leaves when sodium concentrations in the manure extract solutions increased (Appendix, Table 3.3). For example, the leaf sodium concentration of lettuce grown in cow manure extract (10 g/L and 50 g/L) solutions decreased from 4.1 mg/L to 1.3 mg/L as the manure extract solution increased from 12.5 mg/L to 29.6 mg/L. A reduction in leaf salt content may be a salt exclusion strategy (Bartha et al., 2015), while differences in salt tolerance between lettuce cultivars should also be taken into consideration (Bartha et al., 2015).

Calcium

For all manure extract solutions in this study, calcium content increased with an increase in manure concentration. High salt concentrations, including [Na^+], [NH_4^+], [K^+], and [Mg^{++}], can induce calcium deficiency in plants (Adams, 1997; Meharg & Marschner, 2012) and signs of salt toxicity include stunted root and aboveground tissue growth (Bartha et al., 2015). Salt stress may disturb calcium uptake by reducing calcium influx via the plasma membrane and by increasing efflux from plant cells (Bartha et al., 2015). In this study, calcium levels in lettuce tissue were higher when grown in cow manure extract (10 g/L and 25 g/L) solutions than when grown in the control solution (approximately 14 to 15 mg/g dry tissue versus 11 mg/g; Appendix, Table 3.3). At a high concentration of ammonium in all manure extract solutions, a small decrease in calcium was observed in lettuce leaf tissue, and the lowest calcium concentration was recorded in the turkey manure extract (25 g/L) solution. For kale, the calcium content in leaves was always below that of the control solution and a similar trend was observed, where an increase in ammonium in the manure extract solution resulted in decreased calcium content in leaves from plants grown in a higher manure concentration, such as chicken and turkey manure extract solutions containing 25 g/L manure.

The recommended level of calcium in hydroponic systems is approximately 50-150 mg/L (Baudoin et al., 2013). In this study, the only solution that met this recommendation was the control (Hoagland) solution at 159.0 mg/L. Without adequate calcium levels, plants might experience salt stress and might develop blossom-end rot (Adams, 1997). Contrarily, high concentrations of calcium in nutrient solutions appear beneficial to lettuce. In a nutrient solution containing 300 ppm Ca^{++}, leaf and tissue strength increased (Neeser et al., 2005). Therefore, maintaining high calcium concentrations in hydroponic nutrient solutions is preferred.

Magnesium

The magnesium concentration in all manure extract solutions increased with increasing manure concentrations. Earlier studies have shown that the addition of magnesium fertilizer increases magnesium content in lettuce tissues (Holmes & Crowley, 1944). Notably, the amount of magnesium in leaf tissue decreased in kale grown in both chicken and turkey extract solutions, as the manure concentration increased. However, magnesium leaf content increased for kale grown in cow manure solutions as the manure concentration increased. Furthermore, the magnesium content in lettuce leaves decreased in all solutions as the manure concentration increased (Appendix, Table 3.3). Further experiments should be performed to determine if this fluctuation was caused by species-specific salt tolerance or salt stress, particularly inflicted by Na^+ and NH_4^+.

Manganese

At high concentrations, manganese positively affects lettuce and other vegetable plant yields (Cowan, 2006). The highest recorded manganese concentration in this experiment was 0.4 mg/L and this corresponded to the turkey manure extract (50 g/L) solution that also yielded the highest aboveground wet mass. It is important to note that manganese accumulation in kale and lettuce leaves was below any levels that could have caused risk to human health estimated at 2.3 mg/d and 1.8 mg/day for men and women aged 19 years and over, respectively (Przybysz et al., 2017). Manganese cations, Mn^{2+} and Mn^{3+} can act on the central nervous system dependent on the length and intensity of the exposure (Pillay & Jonnalagadda, 2007) (Appendix, Table 3.3). Even if the chicken manure extract solution had a higher manganese concentration than the other manure extract solutions, a high concentration of ammonium might have inhibited growth more than Mn would have benefited plant growth. The optimal manganese concentration in a nutrient solution that maximizes lettuce yield is estimated between 0.1 and 0.5 ppm (Vlamis & Williams, 1973). Therefore, a decrease in ammonium might allow plants to benefit from the other nutrients present in manure extract solutions.

In this study, all plants grown in the chicken manure extract solution containing 50 g/L (50 kg per 10 m^2) manure died before the end of the experiment. The highest plant yield recorded was grown in an extract solution containing 10 g/L (10 kg per 10 m^2) manure, and plant yield decreased at a manure concentration of 25 g/L (25 kg per 10 m^2). A study by Engelbrecht et al. (2012) showed a positive yield for cabbage plants when applied with chicken manure at a rate of 12.5 kg to 25 kg per 10 m^2 (Engelbrecht et al., 2012). Moreover, Munoz et al. (2004) observed an increase in soil-available phosphorous and fruit yield for papayas when 15 kg per 10 m^2 (15 t per ha) of chicken manure was applied (Munoz et al., 2004). Fresh chicken manure can be used when preparing nutrient solutions; however, a preliminary manure-composting period accompanied by the addition of biomass and forced aeration could improve the composition of the nutrient solution while minimizing the presence of harmful elements. Compost preparation and quality assessments have been extensively reviewed in (Bernal et al., 2009).

Other minerals

The concentrations of three metals, copper, iron, and zinc were present in the manure extract solutions at concentrations that were below those suggested by the FAO (Baudoin et al., 2013). It might be necessary to increase these metals' concentrations with different fertilizers or additives. Recent studies identified lettuce as a plant that is sensitive to inorganic contaminants where the half maximal effective concentration (EC_{50}) on root elongation was 49 μM for copper (11.4 mg/L) and 260 μM for zinc (17.0 mg/L) (Lamb et al. 2010). As mentioned above, copper and zinc concentrations in the manure extracts did not exceed 0.8 mg/L and 1.2 mg/L, respectively. Suggested zinc, copper, and iron concentrations for a nutrient solution are 0.13 ppm, 0.03 ppm, and 0.94 ppm (Brechner & Both, 1996). Iron availability in a nutrient solution promotes zinc uptake, while decreasing manganese and cadmium uptake in lettuce (Ys et al., 1991). Therefore, further studies could investigate iron, zinc, and copper uptake in lettuce and kale, using the addition of different nutrients at different concentrations in manure extracts.

3.4 Conclusion

Kale and lettuce growth were attained in all animal manure extract hydroponic solutions, with the exception of chicken manure at 50 g/L. The highest aboveground biomass achieved was lettuce grown with a turkey manure extract solution (50 g/L). All other manure extract solutions resulted in kale yields that were lower than the control Hoagland solution. Based on a PCA analysis, monitoring ammonium, nitrate, potassium, manganese, magnesium, calcium, and sodium levels is critical for preventing nutrient toxicity and deficiency. Monitoring plant growth at higher concentrations of cow and turkey manure may determine an upper limit of manure concentration before reaching toxic levels that inhibit plant growth. The implementation of a pre-processing step, either via composting with additional biomass or forced aeration, could increase nitrate concentrations while reducing ammonium concentrations in manure extracts, consequently preventing ammonium toxicity in chicken and turkey manure extracts.

3.5 Acknowledgement

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), Discovery Grant 355743-13.

3.6 **Appendix**

Table 3.3. Nutrient variation in the leaf tissues. Not a complete sample of all treatments.

Plant	Treatment	Concentration	Na (ug/L)	Ca (ug/L)	Mg (ug/L)	Mn (ug/L)
kale	Chicken	10 g/L	2,357.1	15,805.3	3,516.4	61.9
	Chicken	25 g/L	1,507.0	7,109.4	2,543.8	16.7
	Control		1,072.1	30,461.8	5,168.1	90
	Cow	10 g/L	1,201.0	20,983.6	3,697.2	113.8
	Cow	25 g/L	1,704.2	23,422.1	4,066.1	106.8
	Cow	50 g/L	766.2	23,071.6	6,886.9	91.8
	Turkey	10 g/L	3,574.2	23,790.2	4,746.2	124.3
	Turkey	25 g/L	2,918.2	11,273.9	2,051.3	101.5
lettuce	Chicken	10 g/L	6,290.9	11,279.8	4,539.0	80.1
	Chicken	25 g/L	3,491.5	10,058.5	4,141.9	69.7
	Control		855.6	11,486.2	3,969.8	67.7
	Cow	10 g/L	4,143.6	13,767.2	4,079.7	71.6
	Cow	25 g/L	2,43.04	15,107.5	3,890.5	98.7
	Cow	50 g/L	1,285.5	10,728.8	3,542.9	88.7
	Turkey	10 g/L	4,518.2	10,392.7	3,433.8	93.9
	Turkey	25 g/L	3,629.5	7,982.2	2,380.9	64.9

Mean nutrient content in leaf tissues for sodium (Na), calcium (Ca), magnesium (Mg), and manganese (Mn). These nutrients were selected from the results of the PCA. The control treatment is the Hoagland solution.

Connecting Text

In Chapter 3, the effect of manure extracts was investigated on lettuce and kale growth. Both plants were grown in ebb and flow hydroponic systems with nutrient solutions prepared form chicken, cow or turkey manure. Plant biomasses were assessed to determine the potential of organic fertilizer as a substitute to inorganic fertilizers. Chicken manure, with the highest level of ammonium, was the most toxic, as all plants died at 50 g/L before the end of the experiment. The nutrient analysis isolated six key nutrients to monitor in the solution for optimal growth.

Additional nutrients are required to balance organic fertilizer prepared from animal manure. In Chapter 4, slag cement was added to chicken manure extracts to balance the potassium and calcium content in the solution. An initial experiment helped to determine which of the powder or solid state should be added to the solution to optimize nutrient availability. A second experiment was conducted where kale and lettuce were grown in hydroponic systems with cement and manure extract. Plant biomass was measured and compared to a control, Hoagland, solution prepared from inorganic fertilizers only and which is commonly used in hydroponic solutions.

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4 EFFECT OF SLAG CEMENT ON CALCIUM AND POTASSIUM CONTENT USED IN HYDROPONIC SOLUTIONS MADE FROM CHICKEN MANURE

Abstract

Several studies have investigated techniques to replace inorganic fertilizers with compost teas in hydroponic solutions. However, limited research has been published on the addition of animal manure for hydroponic plant growth. Animal manure is known to be nutrient deficient in K and Ca, while they contain Na which can be toxic to plants at high concentrations. The purpose of this study was to determine if compost teas and slag cement could be linked to improve the nutrient solution and to determine the impact on the growth of Romaine lettuce *(Lactuca sativa* var. longifolia) and Russian kale (*Brassica napus* cv. Red Russian*)*. Five treatment levels of compost tea from chicken manure with slag cement were compared chemically. The lettuce and kale were grown in one of the treatments and their biomass was compared to a Hoagland solution as the control. The addition of dry slag cement powder and cured slag cement blocks increased the Ca (38.8 to 57.4 mg/L), K (93.4 to 121.2 mg/L) and Na (26.3 to 54.5 mg/L) content respectively in the solutions ($p < 0.05$), without reaching toxic levels for Na. Healthy plants were grown in the manure and cement solutions, however they were a fraction of the control treatment, 8% for kale and 19% for lettuce of total aboveground wet mass. Substitution of inorganic fertilizers with a solution made from chicken manure mixed with cement could provide an alternative nutrient solution for plants grown in hydroponic systems once the nutrients are balanced.

Keywords: hydroponic system, slag cement, manure extract, compost tea

4.1 Introduction

Nutrients are essential for plant growth and are delivered to plants through organic and inorganic fertilizers. Within these nutrients, the necessary ions required are referred to as primary macronutrients (N, P and K), secondary macronutrients (Ca, Mg and S) and micronutrients (Zn, Mn, Cu, etc.) (Hernández et al., 2010). Recently, compost teas in agricultural production have been investigated as a replacement to expensive inorganic fertilizers (Carballo et al., 2009; USDA, 2013). Compost teas, a type of compost extract, are watery extracts prepared with compost or compost mixed with manure to cultivate beneficial microorganisms. Chicken manure has been used previously to prepare compost extracts to grow spinach. Results have shown that manure lacks K and Ca ions and has high Na which is known to be toxic for plants at high concentration (Al-Maskri et al., 2010). With the addition of complementary nutrients manure extracts have the potential to substitute inorganic fertilizers (Liu et al., 2016). One method to properly assess the effect of manure extracts on plant growth is with the use of hydroponic systems in a controlled environment as it allows for close monitoring of the nutrients in the solution and minimize the impact of the external environment.

Hydroponic systems are a soilless culture technique used for plant production. With the absence of soil, fertility, erosion and compaction are no longer issues which could impact plant growth. Plants grown in hydroponic systems have better nutritive values and this production method allows for a higher productivity per unit area than field grown agriculture (Surendran et al., 2016; Suvo et al., 2017). Hydroponic systems result in improved water use efficiency and allow for optimized fertilizer management during crop production (Al-Maskri et al., 2010). Use of compost teas in hydroponic systems allows for the conversion of agricultural waste into fertilizer and to meet the nutrient requirements for plant growth at a lower cost (Haghighi et al., 2016). For this reason, additives to compost extracts should be explored in order to balance the nutrient levels.

The application of Portland cement and ground granulated surface blast furnace slag in agriculture has been recently investigated for soil decontamination and stabilization (Kogbara & Al-Tabbaa, 2011). It has been shown that the addition of Portland cement blends to contaminated soils and promotes the fixation and removal of organic contaminants (Paria & Yuet, 2006). Ground granulated blast furnace slag is a geopolymer which is used as an addition to Portland cement mixes and few studies have reviewed its effects on soils and their use as fertilizers. Earlier studies have shown increases in K and Na ions through carbonation of Portland cement (McPolin et al., 2009). Ground granulated burnt furnace slag (GGBS), typically sourced from residues in the steel industry, is known for its high content of Ca oxide and is blended with Portland cement in order to resist to acid (Eloneva et al., 2008). In addition, it has been shown that Portland cement release Na and K at a much slower rate than Ca once in solution (Müllauer et al., 2015; Sajedi & Razak, 2010). These available ions could potentially balance the nutrient solution made from manure extracts.

The objective of this research was to add two forms of slag cement, solid and powder, to chicken manure extract and to measure the effect on Ca, K and Na content over a period of 14 days to optimize the nutrient solution. Based on the results, the solution with highest K and Ca and lowest Na content was used to assess the impact on the biomass of kale and lettuce grown in the hydroponic systems.

4.2 Materials and methods

4.2.1 Cement block preparation

The cement blocks designed for this experiment consisted of a binder mixture of blast-furnace slag (90%) and Portland cement (10%) from Lafarge (Montreal, Qc, Canada). Each block of slag cement consisted of 110 g of powder and 55 g of water; the water-to-binder ratio was of 2:1. The blocks were molded in a conical silicon tray of 570 cm^3 (top radius 7 cm, bottom radius 5 cm and height of 5 cm) and cured for a period of 5 days at 95% humidity (TAO-Tronics Humidifier, TT-AH001, China).

For the second part of the experiments, where plants were grown, the blocks were 6,840 cm^3, proportional to the nutrient solution, so 12 times greater, of the initial 5 L to 60 L (top radius of 27.5 cm, bottom radius of 25.5 cm and height of 3.1 cm)

4.2.2 Manure extracts preparation

Chicken manure was sourced from the Macdonald farm (McGill University, Sainte-Anne-de-Bellevue, Canada). For each solution, aerated manure extracts were prepared with a concentration of 50 g per 1 L of water, following the protocol in Leudtke (2010) and El-Shinawy et al. (1999) (El-Shinawy et al., 1999; Leudtke, 2010). The solution was aerated with an air pump with atmospheric air for 48 hours. The solution was filtered with a 1 mm sieve to remove large fragments and was diluted with tap water to reach a final volume of 5 L with the set concentration. Unused chicken manure was stored in a plastic bag within a plastic container at room temperature.

Sixteen containers were placed in the greenhouse with five different treatments (Table 4.1). Each treatment was repeated three times and the control treatment only once. All treatments with slag cement had the equivalent of 220 g of slag cement powder. For treatment Manure + Cement and Water + Cement (Table 4.1), the slag cement had started its hydration reactions prior to being in contact with manure, when the water was mixed with the slag cement. Same amount of water was added to all treatments (Table 4.1). Manure and water were combined at day 0 (T0). All containers were aerated with an atmospheric air pump. Samples were collected in 50 mL Falcon tubes from each container at day 0 (T0) and 14 (T3) and were stored in a fridge (Frigidaire, model FRT18G6JW0, Electrolux Canada Corp., Mississauga, ON, Canada) at -75^{o}C for a period up to 6 months prior analysis.

4.2.3 Plants growth and harvest

Seeds of red Russian kale (*Brassica napus* cv. Red Russian) (S8422-001, Richters, Goodwood, ON, Canada) and romaine lettuce (*Lactuca sativa* var. longifolia cv. Ridgeline) (Ridgeline Pelleted MT0 OG, Johnny's, Winslow ME, USA) were germinated in pre-washed rockwool rooting medium cubes (Grodan, Roedmond, NL). For each species, 12 cubes were seeded with two seeds and then transferred to a tray. The tray was moved into a growth chamber (CMP4030, Conviron, Inc., Winnipeg, Manitoba, Canada) for two weeks with a constant temperature of 25^{o}C, 50% humidity and 16:8 h of day: night. Distilled water was added when necessary to maintain water level. One week after germination, the seedlings were thinned to one seedling per rockwool cube. The seedlings were grown for two weeks in a growth chamber. The plants were then placed in 10-cm rockwool cubes and transferred to an ebb and flood hydroponic system. Within the hydroponic beds, the seedlings were placed randomly to minimize the edge effect.

All plants were harvested and dried 28 days after being transferred to the greenhouse (42 day at harvest). At harvest, the following characteristics were measured: aboveground wet mass and the number of mature leaves (greater than 2 cm across). Plant samples were dried at 40^{o}C for 5 days and dry mass was measured.

Four ebb and flow recirculating hydroponic systems were used. Each bed was flooded for 15 min every hour, resulting in approximately of 5 cm of water in each bed. One bed served as the control treatment with the modified full-strength Hoagland solution and the other systems had the treatment solutions of chicken manure extracts at 50g/L. Three replicas per plant (lettuce and kale) were grown for the chicken treatment.

Table 4.1. Experiment treatments investigated

Container	**Treatment**
A	Manure
B	Manure + Cement
C	Manure + Cement powder
D	Water + Cement
E	Water + Cement powder
F	Water (Control)

4.2.4 Element measurement

Preliminary step

The elemental analysis of Na, Ca and K was achieved through flame atomic absorption spectroscopy (SpectrAA 220FS, Varian, Palo Alto, CA, USA). Solutions from all treatments were diluted 50 times; 0.2 mL of each treatment was diluted with 9.8 mL of distilled water and replicated 3 times. Lanthanum and cesium were added to each sample (0.1 mL) in order to reduce chemical and ionization interferences (Sanui & Pace, 1968). Each measurement was replicated three times. The standards used for calibration for the Na, K, and Ca were 100 ppm and 50 ppm of their respective ionic solutions. For the K measurements, additional standards were used as readings surpassed the 100 ppm range, the additional standards were 200 ppm and 400 ppm.

4.2.5 Statistical analysis

The statistical analysis was conducted with an open source statistical programming language – Rstudio: Integrated Development Environment for R (Boston, MA, USA). A two-way ANOVA with repeated measures analysis was conducted between the different treatments and cation measurement methods (ISE or flame spectroscopy). The post-hoc comparisons were accomplished using Tukey Honest Significant Difference (Tukey HSD) at a 95% confidence interval.

4.3 **Results and discussion**

4.3.1 Calcium analysis

At time 0, when the manure solution was mixed with the cement, the Ca contents were similar in all treatments, between 15.2-21.1 mg/L, except where cement powder was added (Figure 4.1). In the Manure + Cement powder and Water + Cement powder treatment, Ca content were 37.7 mg/L and 55.4 mg/L respectively. At the end of the experiment, at time T3, the Manure, Water and Water + Cement treatments had similar values, between 17.9-24.6 mg/L. Ca content in the Manure + Cement and Manure + Cement powder increased to 38.8 and 57.4 mg/L respectively. Values for Water + Cement powder treatment decreased to 4.3 mg/L.

The highest change in Ca was at time T3 in the Water + Cement powder treatment, with a decrease of 51.1 mg/L, while the highest increase was in the Manure + Cement treatment with an increase of 22.1 mg/L followed by Manure + Cement powder with 19.7 mg/L ($p<0.05$) (Figure 4.1). In the other treatments, the change in Ca concentration was not statistically significant.

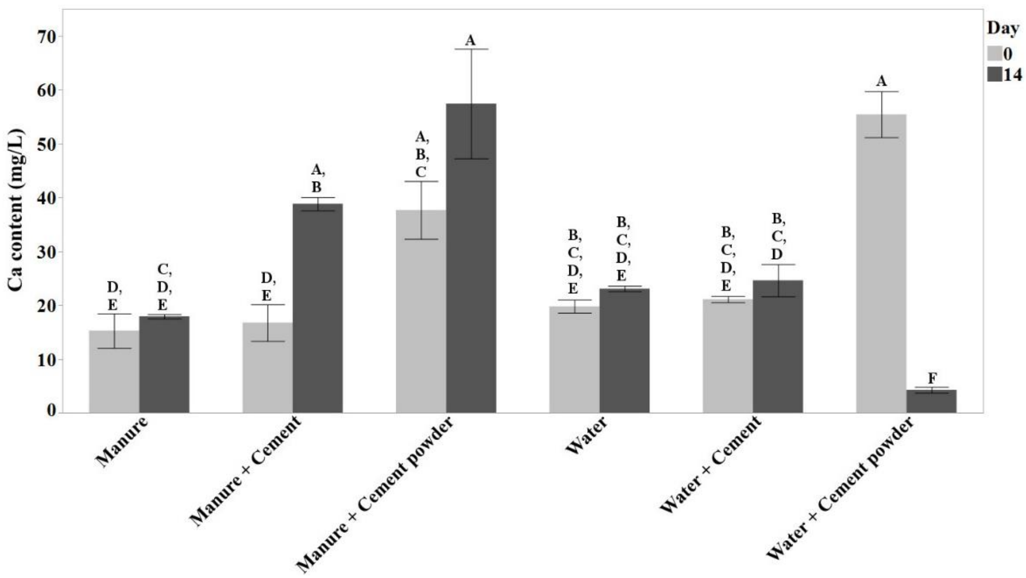

Figure 4.1. Ca content (ppm) for all treatments for T0 and T3. Statistical significance between T0 and T3 are shown by different letters ($p < 0.05$)

4.3.2 Potassium analysis

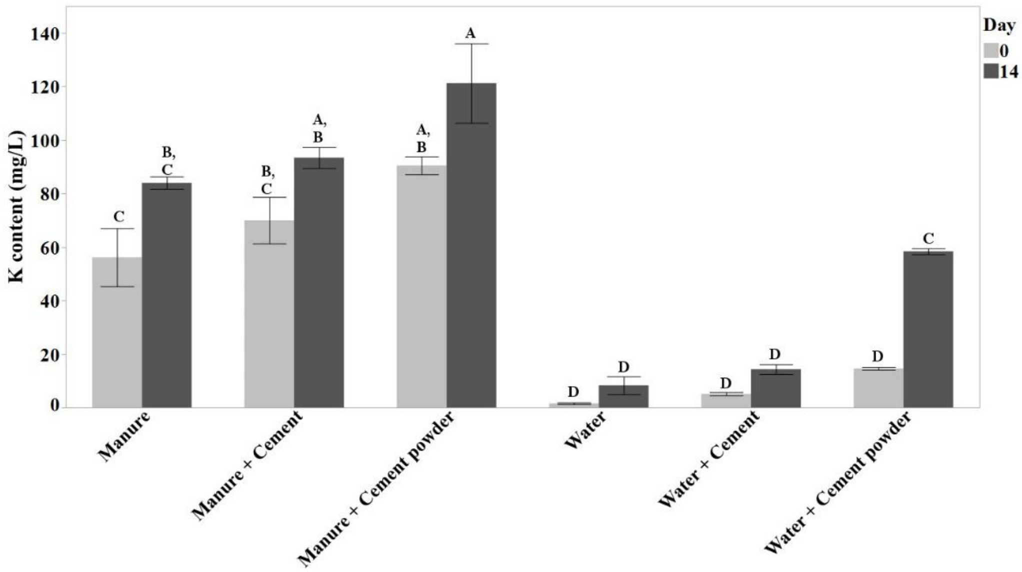

Figure 4.2. Potassium content (ppm) for all treatments at T0 and T3. Statistical significance between T0 and T3 are shown by different letters ($p < 0.05$)

K levels in the water treatments were always below 15.0 mg/L at the beginning of the experiment. K levels in the solutions with manure varied between 56.1-90.5 mg/L. At the end of the experiment, time T3, K content increased in all solutions, but was only significant in the Water + Cement powder treatment, where the concentration went from 14.6 to 58.4 mg/L (Figure 4.2). In the manure solutions, there was an increase of 30-50% in the K content. Highest concentration was in the Manure + Cement powder with 121.2 mg/L followed by Manure + Cement and Manure treatment with 93.4 and 84.0 mg/L respectively.

4.3.3 Sodium analysis

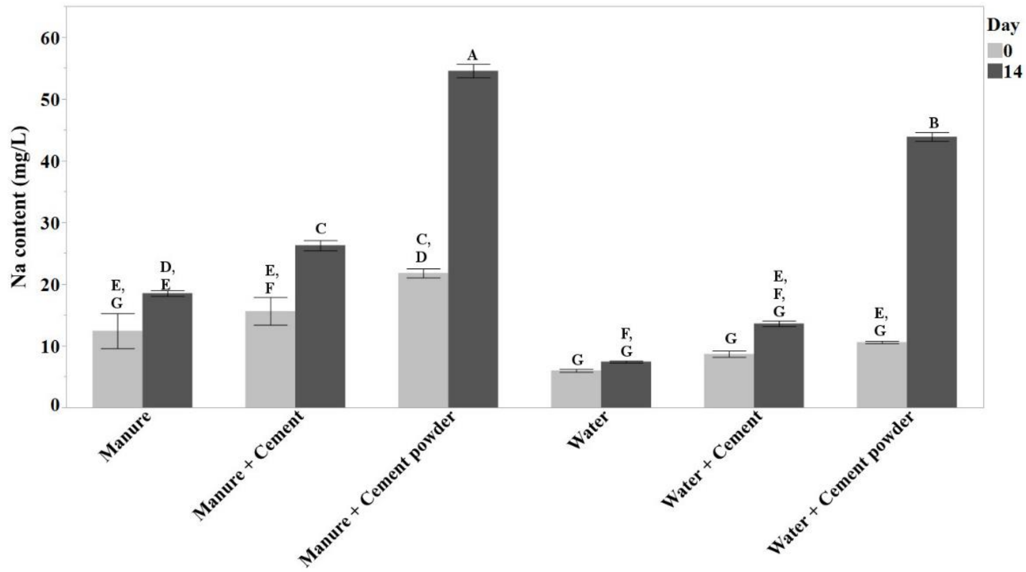

Figure 4.3. Sodium content (ppm) for all treatments investigated at T0 and T3. Statistical significance between T0 and T3 are shown by different letters ($p < 0.05$)

Na content at the start of the experiment, T0, were similar for the water treatments, between 6.0 and 10.6 mg/L. For the manure treatments, Na content was fluctuating between 12.4 and 21.8 mg/L. Highest Na content was measured in the treatments with cement powder, where the Manure + Cement powder treatment had 54.5 mg/L and the Water + Cement powder had 21.8 ppm. Manure treatments had a significant increase in Na content in all their treatments, while this was not the case for the water treatments, where the Water and Water + Cement had similar Na levels at the end of the experiment.

Nutrient release from the cement in the hydroponic solution results from various processes including exchange, decomposition, dissolution or desorption. The rate of release and capacity to release nutrients influences the amount of nutrient supplied in the solution (Barber, 1995). In the Water + Cement solutions, as it was a sterile environment, decomposition could not have occurred. Dissolution of nutrients promoted with aeration could have increased ions availability in the solution.

A decrease in Ca was measured in the Manure + Cement powder treatment. Hydration of Portland cement, at 10% in the mix can cause Ca precipitation once added to water (Escalante et al., 2001). The reactivity of the slag cement powder from Lafarge was greater since it was undergoing its first period of hydration in comparison to the slag cement blocks which had previously cured (Harrisson et al., 1986). An increase in Ca availability in the manure treatments over time when the cement was added could have been possible as organic matter, such as fluvic and organic acids inhibit $CaCO_3$ precipitation (Lin et al., 2005). $CaCO_3$ was not measured in the solutions and cannot be confirmed. In addition, Ca solubility decreases with a lower pH and further investigation could explain if the Ca variation in the water treatment was caused by the pH levels.

Manure + Cement and Manure + Cement powder solution seemed to be the most optimal solution for plant growth. After a period of two weeks, the highest Ca content was in the Manure + Cement powder at 57.4 mg/L, however the highest increase in available Ca was in the Manure + Cement solution, an increase of 22.1 mg/L reaching 38.8 mg/L (Figure 4.1). Similar trends were observed for K and Na, where the highest ion content was recorded in the Manure + Cement powder treatment followed by the Manure + Cement treatment (Figure 4.2 and Figure 4.3). Aeration of the solutions promotes composting. Mineralization during composting allows for an increase in nutrient availability in the manure solutions and explains the higher K and Ca content after the period of 2 weeks (Eneji et al., 2003). A study by Tiquia and Tam (2002) on chicken manure composting showed an increase in Cu, Zn, P, K and NO_x^- N over a period of 128 days (Tiquia & Tam, 2002). An increase in nutrient levels was explained by the loss of organic matter (C, H, N and O) as CO_2 and H_2O during composting. Compost stability can be reached in 15 days but can extend up to180 days depending on the treatment and other factors including the nature of the organic material, pile size, aeration frequency and composting method (Tiquia & Tam, 2002). Eneji et al. (2002) showed that the chemical composition of manure will influence the nutrient released and the rate at which it will be released. In addition, soil type influences nutrient availability (Eneji et al., 2003). The use of solid cement and powder cement may have influenced the mineralization of other nutrients in the solution when mixed with manure.

The highest increase in Na was found in the manure treatment with cement powder. The increase in Na increased over time due to the hydration of the Ca silicates of the slag Portland cement (Pacheco-Torgal et al., 2008). A previous study reported that Na ions found in the cement powder can be replaced with Ca ions (Hong & Glasser, 1999). As the cement powder was unreacted at T0, the treatments with powdered cement underwent hydration in the water and manure solutions. Ca and Na reaction in the solution might have reduced the Na available in the manure solutions. One main challenge using a hydroponic system is the accumulation of salt in the system over time (Al-Maskri et al., 2010). This can generate salinity stress at 50 mM and 100 mM (1,150 and 2,300 ppm respectively) on plant growth (Al-Maskri et al., 2010). Optimal Na content in hydroponic systems is set around 230 ppm (Baudoin et al., 2013). Long-term irrigation with low salt content (5 mM or 115 ppm) was shown to increase β-carotenoid content by 80% and lutein content by 37% in romaine lettuce (*Lactuca sativa* L.) grown in soil without having a negative effect on yield or visual crop characteristics (Kim et al., 2008). Lutein is known as an eye health promoter, anticolon cancer agent, and antioxidant, while β-carotenoid is an important antioxidant (Kim et al., 2008). The reduction of Na in manure compost teas could be beneficial to romaine lettuce and reduce osmotic pressure on plant root system.

4.3.4 Plant growth

Tukey HSD test was performed to compare the aboveground wet and dry mass for kale and lettuce in the chicken and control, Hoagland, solutions. The chicken manure treatment with cement and control treatments were replicated three times. Lettuce and kale in the control treatment were significantly different than the lettuce and kale grown in the chicken manure.

Smaller plants were grown in the chicken manure treatment with solid cement blocks ($p < 0.05$) (Figure 4.4). Average aboveground wet mass for kale in the chicken solution was 8% of the control treatment, only 4.4 g, while it was 50.2 g in the Hoagland solution. As for the lettuce, it was 19% of the control treatment, with 18.1 g and 95.2 g respectively. Even if the plants were smaller, they did not show sign of nutrient toxicity (Figure 4.6).

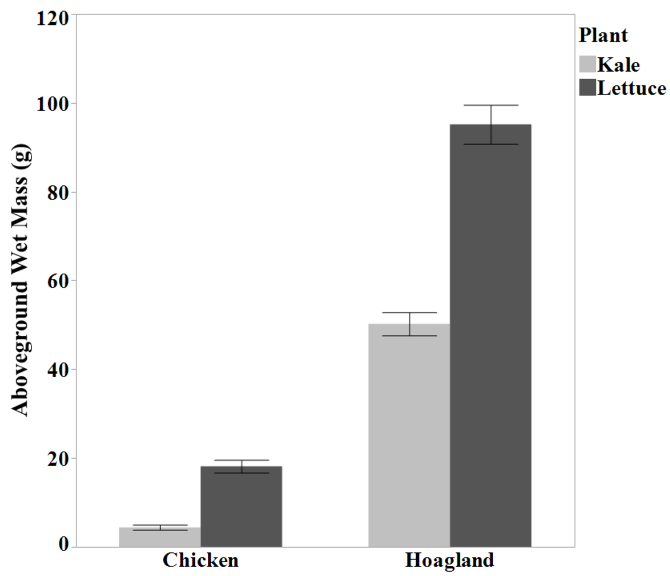

Figure 4.4. Aboveground wet mass for kale and lettuce. Statistical significance between the chicken and Hoagland solution were measured for both plants ($p < 0.05$)

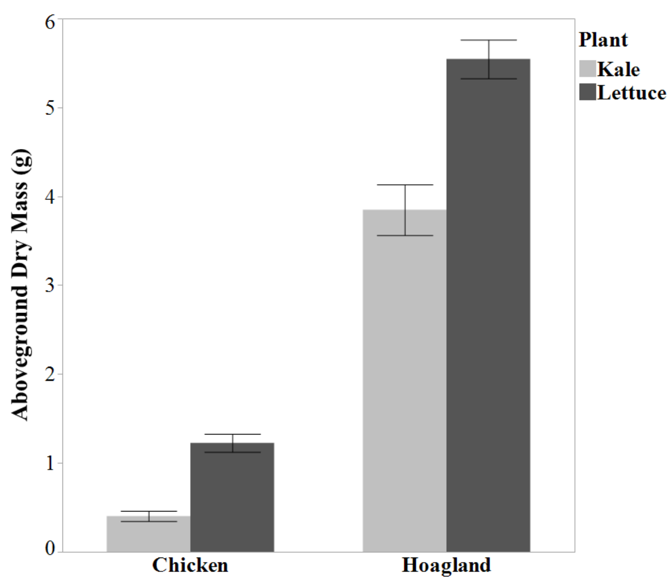

Figure 4.5**.** Aboveground dry mass for kale and lettuce. Statistical significance between the chicken and Hoagland solution were measured for both plants ($p < 0.05$)

Aboveground dry mass for kale was 10% of the Hoagland solution, 0.4 g for the chicken solution, while it was 3.8 g in the Hoagland solution (Figure 4.5). Lettuce dry mass was 22% of the Hoagland's mass, being 1.2 g in the chicken treatment and 5.5 g in the Hoagland solution.

Table 4.2. Leaf tissue nutrient content

Sample	Treatment	Ca Mean ± SE	K Mean ± SE	Na Mean ± SE
Kale	Manure + Cement	5.8 ± 0.7*	5.6 ± 0.1	3.0 ± 0.1*
	Control	19.1 ± 0.4*	5.8 ± 0.1	0.8 ± 0.0*
Lettuce	Manure + Cement	3.0 ± 0.3*	5.3 ± 0.2*	4.9 ± 0.1*
	Control	5.5 ± 0.9*	6.7 ± 0.0*	0.2 ± 0.0*

Leaf tissue analysis showed significant difference in the tissue levels for kale and lettuce (Table 4.2). Statistical results, identified by *, showed an effect on all three nutrients for lettuce, but only for Ca and Na for kale. K levels in kale were similar in both solutions. Similar K values were also measured for lettuce, but since the standard error was very small, it was significant ($p < 0.05$).

Table 4.3. Solutions nutrient content

Treatment	Day	Ca	K	Na
Manure + Cement	0	40.0	213.0	33.3
	28	26.7	530.0	965.0
Control	0	270.0	230.0	10.0
	28	350.0	290.0	23.0

Preliminary nutrient analysis of the chicken and control solution where the plants were grown showed a decrease in Ca, in the chicken solution, but an increase in the control solution

(Table 4.3). K content increased in both solutions and Na had more than a 10-fold increase in the chicken solution.

Figure 4.6. Lettuce and kale at harvest in chicken & cement (left) and Hoagland solution (right)

Healthy kale and lettuce plants were grown in the Manure + Cement solution, however they were significantly smaller than the control treatment (Figure 4.6). Aboveground wet and dry mass of lettuce was 19% and 22% respectively of the control treatment, and was 8% and 10% for kale (Figure 4.4 and Figure 4.5). Even if the plants did not show signs of nutrient toxicity, they were under nutrient stress in the Manure + Cement treatment. Animal manures are known to be rich in salt contents and can inhibit plant growth if applied at high rates in soil (Walker & Bernal, 2008). In this experiment a hydroponic system was used, where the salt content increased as it was recirculated in the system and there was no loss possible through percolation. In addition, the nutrient solution was not changed over the 28 day period, however a study completed by El-Shinawy et al. (1999) using chicken manure in a nutrient film technique replaced the solution every 7 days (El-Shinawy et al., 1999). This method could have decreased nutrient deficiency, such as Ca that decreased over time in the solution (Table 4.3).

Leaf tissue analysis showed that high Na levels were absorbed by both plants, 3 mg/g for kale and 4.9 mg/g for lettuce. The nutrient solution was also very high in Na, especially at the end of the experiment with 965 mg/L (Table 4.2 and Table 4.3). In the Manure + Cement solution Ca was deficient as the concentration in the solution decreased over time, from 40 mg/L to 26 mg/L. In addition, even if lettuce Ca leaf content were close to the control, 3.0 mg/g for the treatment and 5.5 mg/g for the control, this was not the case for kale, where there was close to 4 times the Ca content in the control plant leaves (Table 4.2). The observed increase of K and Na in the Manure + Cement solution was caused by the water evaporation from the solutions. Similar yet significant difference of K was measured in lettuce leaves, 5.3 mg/g for the Manure + Cement and 6.7 mg/g for the control. Growing plants in a rich Na environment, as the Manure + Cement treatment, can inhibit growth as it can negatively impact gas exchange, photosynthesis and protein synthesis (Walker & Bernal, 2008). Plants grown in highly saline soils increase Na and Cl leaf tissue content and can inhibit uptake of Ca, K, N and P. In this study, the lack of Ca in plant tissue was measured and the Manure + Cement solution decreased in Ca over time. Target Ca content for lettuce is around 292 ppm, 242 ppm for K and 230 ppm for Na (Baudoin et al., 2013). K leaf levels were only slightly lower than the control treatment, however the Na levels were higher for both plants in the Manure + Cement solution. The addition of supplemental Ca could fix this issue, however the prevention of Na accumulation should be investigated.

4.4 Conclusion

Addition of solid cement and cement powder to chicken manure can improve its nutrient composition for plant growth. An increase in K, Ca and Na was measured in all the solutions where manure was mixed with the cement. To minimize Na availability, lettuce and kale were grown in the Manure + Cement solution. Even if healthy plants were grown, their size was significantly smaller than the control treatment, showing signs of nutrient deficiency. With the addition of cement, the values of K and Ca were closer to the optimal levels for plant growth, however complementary fertilizers are required to create a balanced hydroponic solution.

4.5 Acknowledgement

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Connecting Text

In Chapter 4, slag cement was added to chicken manure extract solutions used for plant growth in hydroponic systems. An initial experiment showed an increase in the potassium, calcium and sodium content when powder cement was added to the manure extract. To minimize sodium accumulation in the solution the solid cement was added to the manure extracts. Healthy lettuce and kale were grown in the chicken manure extract with solid cement. However, they were smaller than the Hoagland solution, showing signs of nutrient imbalance.

Following the study on the addition of slag cement to balance the nutrients in the manure extracts, it became apparent that ammonium content is very high and needs to be reduced. In Chapter 5, the primary objective was to reduce ammonium content in the manure extract as it is toxic to plant and can limit growth. The secondary objective was to increase nitrate content, which is the preferred form of nitrogen for plants. The study looked at the impact of aeration and the addition of molasses in manure extract for 12 days. Ammonium and nitrate were monitored with ion-selective electrodes during the experiment and values were adjusted with a regression equation to the Lachat readings.

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5 AERATION AND THE ADDITION OF MOLASSES TO A HYDROPONIC SOLUTION MADE FROM CHICKEN MANURE TO CONTROL AMMONIUM AND NITRATE CONTENT

Abstract

Compost teas made from chicken manure are being used in hydroponic systems, however high ammonium content can inhibit plant growth. In this study, aeration of chicken extract solution promoted nitrification and resulted in a reduction of ammonium content by 62% within a period of 12 days. Molasses was added to promote denitrification, a biological process of reducing NO_3 or NO_2 to N_2O or NO where the addition of a carbon source to the solution can promote the growth of denitrifying bacteria. However, the nitrate levels were still minimal, below 10 mg/L, at the end of the experiment. Ion-selective electrodes were used to monitor ammonium content in the solution and readings were adjusted with a simple linear regression model to be between 2-16% above the Lachat measurements. Nitrate concentration was too low for the ISE to be accurately monitored.

Keywords: manure extract, hydroponic systems, organic fertilizer

5.1 Introduction

Hydroponic systems are a soilless culture technique used for plant production and are usually present in controlled environments such as greenhouses. Hydroponic systems do not use soil, removing issues of soil fertility, soil erosion and compaction. Plants grown in hydroponic systems are known to have better nutritive values and this production system allows for a higher productivity per unit area than field grown agriculture (Surendran et al., 2016; Suvo et al., 2017). These systems have improved water use efficiency and improved fertilizer management during crop production.

Compost teas, or water extracts, made from compost or animal manure are becoming more popular as they can stimulate plant root and vegetative growth, increase crop yield and quality (Pane et al., 2016; Scheuerell & Mahaffee, 2000). Compost teas are rich in effective microbes (EM) that are known to improve yield and quality of vegetable crops (Daly & Stewart, 1999). Manure is also a known source of EMs and could reduce the cost of compost tea production (Ch'ng et al., 2013). Other materials can be added to the compost teas to stimulate plant-beneficial microbial population, such as molasses (Duffy et al., 2004). Previously, compost extracts have been tested for vegetable growth in hydroponic systems and the plants have been monitored for their nutritional content and microbes measured for N_2-fixation, denitrification, sulfate reduction and phosphorus solubilization (Janzen et al., 1995). One main drawback of the use of animal manure is the presence and growth of human pathogenic bacteria when molasses is added to the solution. Studies showed an increase of *Salmonella enterica* serovar Thompson and *Escherichia coli* O157:H7 when molasses was added to the solution made from both chicken and cow manure (Duffy et al., 2004). However, this increase was not measured in compost extracts without molasses.

Excess manure used in agricultural fields can result in leaching to surface water and groundwater where it can cause eutrophication as it contains high content of phosphorus and nitrogen which promotes algal blooms, aquatic weeds, and leads to fish dieback and loss of biodiversity (Jamieson et al., 2003). However, if properly managed, the excess nitrogen can be transformed to forms available for plant growth via ammonification and nitrification. Nitrification is the formation of nitrite (NO_2-N) and nitrate (NO_3-N) from ammonium (NH_4) promoted by biological activities in an aerobic environment (Alexander, 1977; Reddy & D'angelo, 1997). Nitrate (NO_3-N) is the preferred source of nitrogen for plants while nitrite (NO_2-N) can be toxic for plants (Shinohara et al., 2011). Nitrifying bacteria are slow growing and require high oxygen demand, greater than 1-2 mg/L (Cottingham et al., 1999; Hammer & Knight, 1994). Therefore, the use of supplemental aeration to increase the dissolved oxygen level is necessary for the promotion of nitrification (Jamieson et al., 2003).

Denitrification is the biological process of reducing NO_3 or NO_2 to N_2O or NO and can be promoted by the addition of a carbon source to the solution to promote denitrifying bacteria (Quan et al., 2005). Molasses, a by-product of sugar production and constitutes 48-50% of sugar, is a good source of carbon that could be used for denitrification purposes (Quan et al., 2005). Preliminary studies using molasses and waste measured an increased growth on onion, pea and sweetcorn by 29%, 31% and 23% respectively (Daly & Stewart, 1999). Molasses added in the treatments were used as a source of carbohydrate for the effective microbes present in the chicken manure (Ch'ng et al., 2013). This method can be used to control nitrate in a high nitrate environment (Cottingham et al., 1999). However, N_2O release should be monitored as N_2O produced by microbial processes of denitrification and nitrification is a known greenhouse gas and an ozone depleting substance and (Domeignoz-Horta et al., 2016).

Most common method to monitor nutrient levels in hydroponic systems are with a pH probe and an EC meter, however these instruments do not provide information on the concentration of individual ions, which makes it difficult to balance the nutrient solution as they are depleted over time (Kim et al., 2013).

The use of ion-selective electrodes (ISE) allows for the measurement of the individual ions (Kim et al., 2007). ISEs are potentiometric sensors that responds to the activity of certain ions (Radu et al., 2013). An ISE has a selective membrane that separates an internal reference solution and the analyte and measures a potential difference at the solution-membrane boundary with a high-impedance voltmeter (Radu et al., 2013). ISEs are becoming more common and tend to replace laboratory measurements as they are portable, allow real-time measurement of samples, have a wide dynamic range and are easy to use (Kim et al., 2007).

The objective of this study was to measure the effect of molasses added to chicken manure tea on ammonia and nitrate concentration over a period of 12 days with different molasses concentration. Measurements were compared between Lachat flow injection autoanalyzer and ISEs readings.

5.2 Materials and methods

5.2.1 Manure extracts preparation

Chicken manure was sourced from the Macdonald farm, McGill University, Sainte-Anne-de-Bellevue, Canada. For each solution, aerated manure extracts were prepared with a concentration of 50 g per 1 L of water (El-Shinawy et al., 1999; Leudtke, 2010). The solution was aerated with an air pump with atmospheric air for 48 hours. The solution was filtered with a 1 mm sieve to remove large fragments and was diluted with tap water to reach a final volume of 4 L with the set concentration. Unused chicken manure was stored in a plastic bag within a plastic container.

Twelve containers were placed within the university's greenhouse with different treatment (Table 5.1). Each treatment was repeated three times. Blackstrap molasses was sourced from a local grocery store (Wholesome Sweetener, Organic Blackstrap Molasses, 472 mL). Molasses was added to the different treatments at day 0. All containers were aerated with an atmospheric air pump (Marina 200, Rolf C. Hagen Ltd, UK, West Yorkshire). Samples were collected at day 0, 4, 8 and 12 and stored in a fridge until ammonium and nitrate analysis was performed.

Table 5.1. Manure concentration in treatments

Level	**Treatment**	**Molasses concentration (% V/V)**
A	Manure only	0
B	Manure with molasses	0.5
C	Manure with molasses	1
D	Manure with molasses	1.5

5.2.2 Element measurement

Ammonium and nitrate measurement in solution

Nutrient solutions were let to sediment naturally for a period of a week in the fridge. Then for NH_4 analysis, solutions were diluted 1,000 times. NH_4 in the sample reacted to a buffer solution prepared with phosphate, salicylate and hypochlorite. The reaction was amplified by heating the solution to 60°C. The green color of the solution was measured colorimetrically at 660 nm on a Lachat flow injection instrument (Lachat Instruments, Quickchem, Milwaukee, WI, USA) (Lachat Instruments, 2008).

For NO_3, samples were not diluted prior to analysis as the content was below 10 ppm. NO_3 concentration was determined taking the solution and passing it through a copperized cadmium column (Cu-Cd column) that resulted in a reduction step to transform the NO_3 into NO_2 (nitrite) (Otsuki, 1978). The nitrites combined with the color reagent of sulfanilamide and phosphoric acid and resulted in a magenta color. The solution was measured colorimetrically at 520 nm (Lachat Instruments, 2008) with a Lachat flow injection instrument (Lachat. Quickchem, Milwaukee, WI).

5.2.3 Plant growth

Based on findings, a preliminary experiment was conducted to see the detrimental effect of NH_4 on plant growth. Lettuce and kale were grown in an ebb and flow hydroponic system using Hoagland solution as the control treatment and a modified solution to simulate NH_4 toxicity. KOH, $(NH4)_2PO_4$ and $CaCl_2$*$2H_2O$ were used to replace KNO_3 and $Ca(NO_3)_2$*$4H_2O$ in the Hoagland solution.

Seeds of red Russian kale (*Brassica napus* 'Red Russian') (S8422-001, Richters, Goodwood, ON, Canada) and romaine lettuce (*Lactuca sativa* var. longifolia) (Ridgeline Pelleted MT0 OG, Johnny's, Winslow ME, USA) were germinated in pre-washed rockwool rooting medium cubes (Grodan, Roedmond, NL). For each species, 12 cubes were seeded with two seeds and then transferred to a tray. The tray was moved into a growth chamber (CMP4030, Conviron, Inc., Winnipeg, Manitoba, Canada) for two weeks with a constant temperature of 25^{o}C, 50% humidity and 16:8 h of day: night. Distilled water was added when necessary to maintain water level. One week after germination, the seedlings were thinned to one seedling per rockwool cube. The seedlings were grown for two weeks in a growth chamber. The plants were then placed in 10-cm rockwool cubes and transferred to an ebb and flood hydroponic system. Within the hydroponic beds, the seedlings were placed randomly to minimize the edge effect.

All plants were harvested and dried 28 days after being transferred to the greenhouse (42 days old at harvest). At harvest, the following characteristics were measured: aboveground wet mass and the number of mature leaves (greater than 2 cm across). Plant samples were dried at 40^{o}C for 5 days and dry mass was measured.

Four ebb and flow recirculating hydroponic systems were used. Each bed was flooded for 15 min every hour, resulting in approximately 5 cm of water in each bed. Two beds served as the control treatment with the modified full-strength Hoagland solution and the other two systems had the modified solutions.

5.2.4 Ion-selective electrodes

Direct potentiometry was selected over one point calibration, incremental techniques and multiple sample addition, as this method of analysis is the most useful when the samples have a wide range of concentration (Toledo, n.d.). Two ISE were used: detectION ® 3021 Nitrate and detectION 3051 Ammonium Combination (Nicosensors, Huntingdon Valley, PA, USA). Both sensors were plastic membrane sensor with a double chamber reference system. A dissolved oxygen probe (Atlas Scientific LLC, Long Island City, NY, USA) was used to determine the percent oxygen saturation of the solution.

Calibration curves were prepared for both NO_3 and NH_4 ISEs. For both electrodes, the electrode potentials developed were measured for known stock solution of 10, 100, 500 and 1,000 ppm for NH_4, and 10, 100 and 500 for NO_3. For NO_3, the stock solution was prepared with KNO_3, and NH_4Cl for NH_4. For each reading, 5 mL of sample or stock solutions were prepared in which the tip of the ISE was placed. Ionic strength adjustment buffer (ISAB) were added to the stock solution and samples before measurement. For samples where NH_4 was measured, 1 M $MgSO_4$ were added at 10% v/v, as for NO_3, 2 M $(NH_4)_2SO_4$ was added at 2 mL per 100 mL of sample (Toledo, n.d.). ISEs were first calibrated using the standards solution, from the lowest to highest concentration, then the manure samples were measured. The values for the standards solutions were used to determine the calibration equation and to determine the concentration of NO_3 and NH_4 in the solutions.

For the D.O. probe calibration, the probe was calibrated while being first exposed to the air for a period of 30 seconds. A small amount of water movement is recommended by the manufacturer. During this experiment, 300 mL were taken from the treatment and was stirred manually, and measurement were made within the first minute.

5.2.5 Statistical analysis

JMP version 13.0.0 (SAS, Cary, NC, USA) was used analysis of repeated measure (MANOVA) between the different treatments and time effect for NH_4, NO_3 and D.O. ISE and Lachat measurements were compared to determine any offset.

5.3 Results

5.3.1 Effect of ammonium

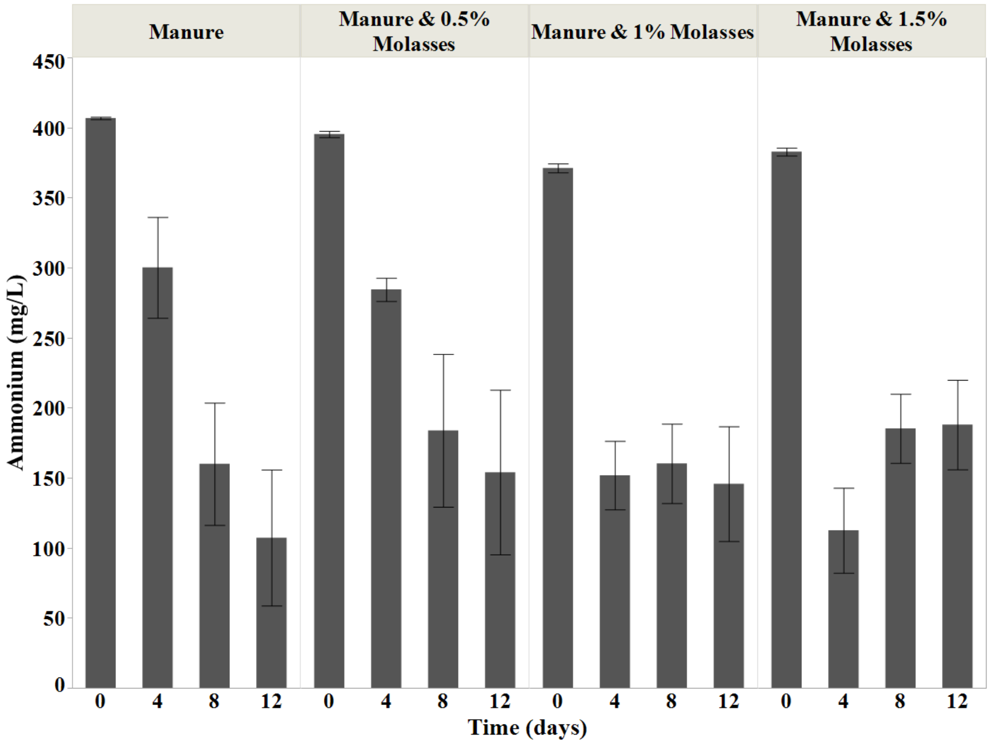

Figure 5.1. Ammonium content (mg/L) for all treatments over time.

For ammonium content, the Sphericity Test was not statistically significant ($p=0.051$), therefore sphericity assumption was not violated and the Univariate unadjusted Epsilon test was used to determine if there was an effect of treatment and time. Statistically significant results were measured for the time effect ($p < 0.0001$) and time*treatment effect ($p < 0.0017$), however there was no statistical significance for the treatment effect ($p = 0.5541$).

Overall mean ammonium content in all treatments decreased over time from 389.5 mg/L at the start of the experiment to 148.7 mg/L at the end. The fastest response was recorded for the Manure & 1.5% Molasses treatment and Manure & 1% Molasses treatment, where the ammonium content dropped from 382.8 to 112.5 mg/L and 371.2 to 151.8 mg/L respectively within 4 days (Figure 5.1). Ammonium content increased in both treatments back to 145.7 and 187.8 mg/L for

Manure and 1% Molasses and Manure & 1.5% Molasses respectively at the end of the experiment, day 12.

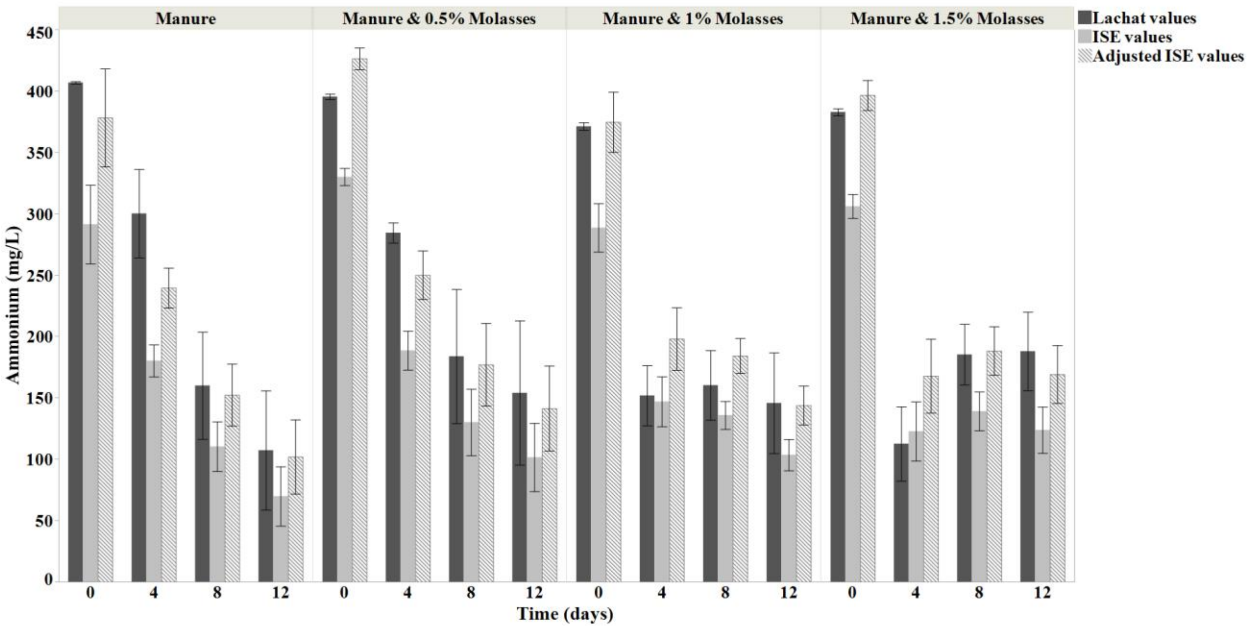

Figure 5.2. Measured and adjusted values recorded (mg/L) for ammonium ISE with in comparison to Lachat values.

A simple linear regression was used to model the relationship between the Lachat and ISE readings for ammonium (Figure 5.2). Difference, in percent, from the Lachat readings and ISE measurements varied from -14.5 to -28.0% before adjusting the values. A linear fit was used to adjust the values (Equation 2) with a $R^2 = 0.87$. After the transformation, the difference between the Lachat and the adjusted ISE values reduced being from 1.6 to 15.7%. Second degree polynomial equation was tried, with an $R^2 = 0.91$, however the difference from the Lachat values were greater, with a difference fluctuating from -22.4% to 17.3% and were discarded. A simple linear regression was also used to model the relationship between the Lachat and the ISE readings with ISAB added to the samples for ammonium, however, the linear fit was weaker with a $R^2 = 0.79$ and was discarded.

Equation 2. Linear fit equation to adjust the values of the ISE readings for ammonium

$$NH_4\ Flame\ value = 14.8435 + 1.2470 * NH_4 ISE\ value$$

5.3.2 Effect on nitrate

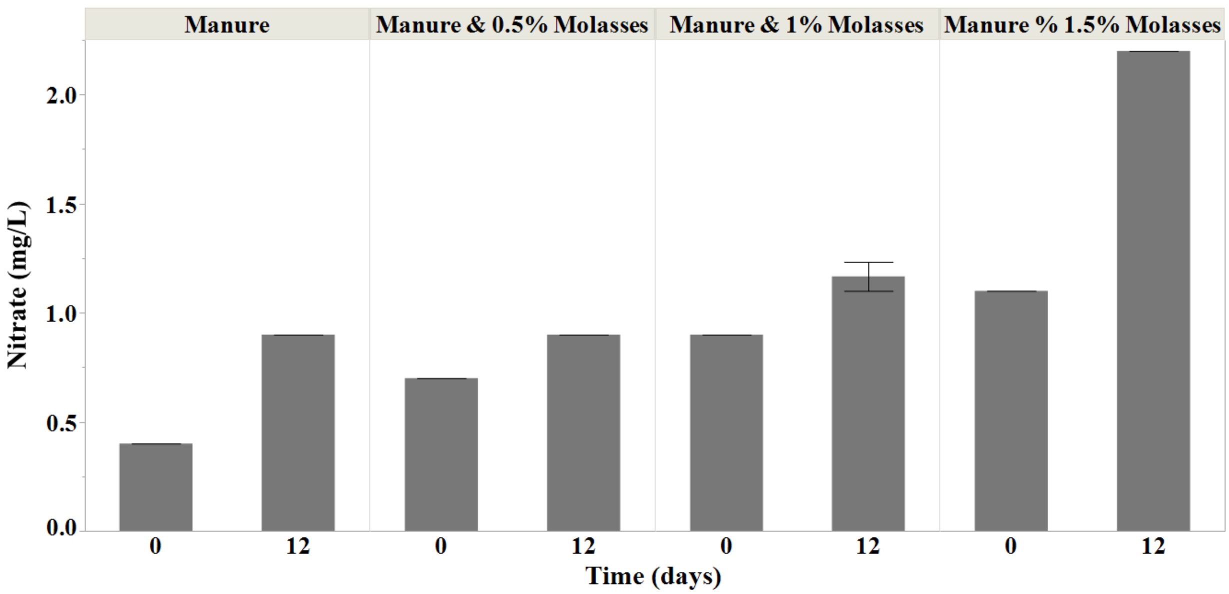

Figure 5.3. Nitrate content (mg/L) for all treatments sampled at the beginning and end of experiment.

For the nitrate content, statistically significant results were measured for the time effect ($p < 0.0001$) and time*treatment effect ($p < 0.0001$ and treatment effect ($p < 0.0001$). Since there were only two levels (day 0 and 12), sphericity is always met and was not evaluated (Hinton et al., 2004).

Overall mean nitrate content in all treatments increased over time from a minimum of 0.4 mg/L at the start of the experiment to a maximum of 2.2 mg/L (Figure 5.3). Greatest increase in ammonium content was measured in the Manure & 1.5% Molasses treatment, where the nitrate content doubled from 1.1 mg/L to 2.2 mg/L. Manure only and Manure & 0.5% Molasses had a 0.9

mg/L content of nitrate at the end of the experiment, while it was 1.2 mg/L for Manure & 1% Molasses.

Table 5.2. Regression equations for time and molasse addition.

Nutrient	Equation	R^2	RMSE	ANOVA Prob>F
Ammonium	$NH_4 = 363.6746 - 19.02 * time - 25.2883 * Molasse + (time - 6) * ((Molasse - 0.75) * 8.6895)$	0.606	74.875	<0.0001*
Nitrate	$NO_3 = 0.29 + 0.6467 * molasse + 0.0431 * time + (molasse - 0.75) * ((time - 6) * 0.0311))$	0.851	0.209	<0.0001*

Best fit model for ammonium was with $R^2 = 0.606$ (Table 5.2). Time and time*molasses effect was significant for ammonium, but was not the case for molasses only. When molasses was removed from the model, the time*molasses effect became non-significant and the ANOVA test was not significant. For nitrate, all effects were significant and had a model with $R^2 = 0.851$.

5.3.3 Nitrate ion-selective electrode

Nitrate concentrations were measured with ISE, but the values were below the threshold of measurement for the ISE at 10 ppm at day 12 and below 1 ppm at day 0.

5.3.4 Dissolved oxygen levels

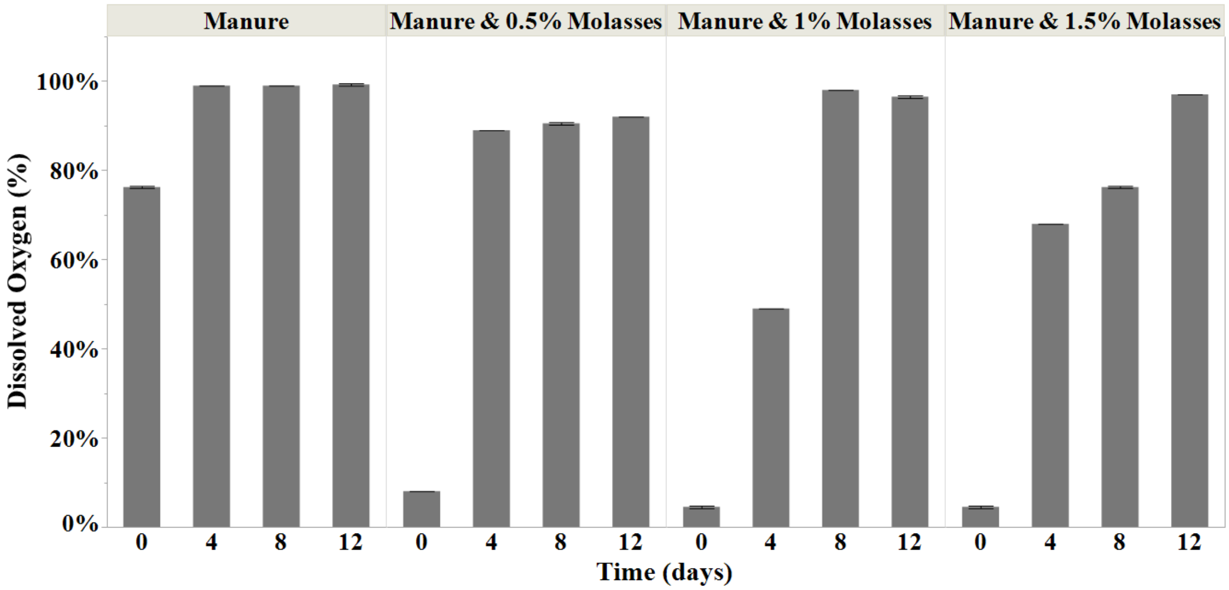

Figure 5.4. Dissolved oxygen content (%) for all treatments over the experiment.

For the dissolved oxygen content, the Sphericity Test was significant ($p < 0.05$), therefore sphericity assumption was violated. The Univariate unadjusted Epsilon test was used to determine if there was an effect of treatment and time as the results were significant. Statistically significant results were measured for the time effect ($p < 0.0001$), time*treatment effect ($p < 0.0001$) and treatment effect ($p < 0.0001$).

Over time, the dissolved oxygen levels went from 23.3% to 96.2% in all treatments (Figure 5.4). In the Manure only treatment, the initial D.O. level was 76.3% and increased to 99.3% at the end of the experiment. In all treatments where molasses was added, the D.O. levels went as low as 4.5%, for Manure & 1% Molasses and Manure & 1.5% Molasses, at the beginning of the experiment to 96.5 and 97.0% respectively at the end of the experiment.

5.3.5 Preliminary plant yield

Preliminary results on the effect of substituting NO_3 with NH_4 in the Hoagland solution on plant growth showed a significant impact on both the aboveground wet and dry mass (Figure 5.5). For kale, the Hoagland treatment had over 6 times the aboveground wet mass, 19.7 g for Hoagland and 3.2 g for NH_4 treatment, and over 5 times the aboveground dry mass, 1.4 g and 0.2 g respectively. For lettuce, the aboveground wet mass was close to four times higher in the control, 29.2 g in the Hoagland and 7.4 g in the NH_4 treatment, and 2.5 times for the aboveground dry mass, 1.5 g and 0.6 g respectively. However, precipitation of nutrients was observed in the modified NH_4 treatment (Figure 5.6) after the beginning of the experiment. Even at the end of the experiment, the precipitates were still present.

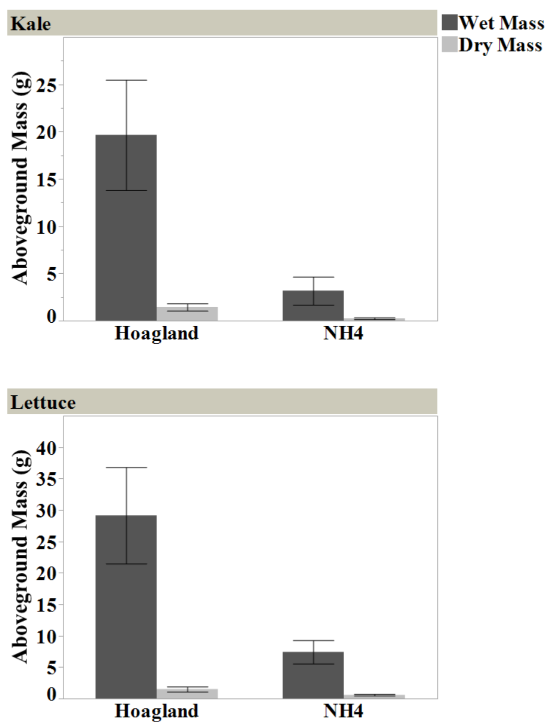

Figure 5.5. Aboveground wet and dry mass for kale and lettuce in the different treatments.

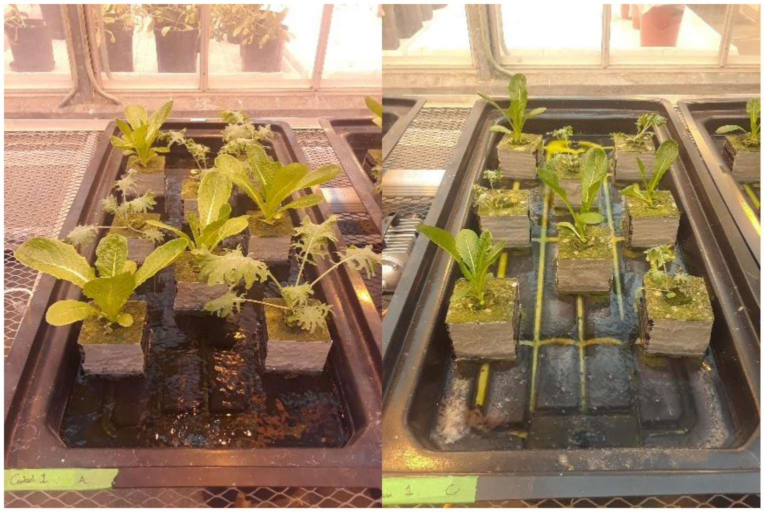

Figure 5.6. Lettuce and kale at harvest in the Hoagland solution (left) and in the modified solution (right).

5.4 Discussion

High NH_4 content is known to be a limiting factor in plant growth and should be minimized (Britto et al., 2001; Daly & Stewart, 1999). In all treatments, NH_4 decreased whereas NO_3 content increased over time suggesting that mineralization from NH_4 to NO_3 occurred (Ch'ng et al., 2013). At the beginning of the experiment, mean NH_4 concentration in the four treatments were 382.8 mg/L and reduced to an average of 148.7 mg/L within 12 days (Figure 5.1). In an aerated environment, ammonia oxidation occurs and results in the increase of nitrate (Quan et al., 2005). Similar results were measured where aeration and mixture of hydrolyzed molasses increased NO_3 concentration (Quan et al., 2005). The addition of molasses at different concentration accelerated NH_4 reduction within the first 4 days in the Manure & 1% Molasses and Manure & 1.5% Molasses, where NH_4 content decreased from 371.2 to 151.8 mg/L and to 382.8 to 112.5 mg/L respectively. The addition of molasses influenced NH_4 mineralization rate and would explains why there is a significant time * treatment effect observed. This effect also increased oxygen demand.

When molasses was added to the treatment, the dissolved oxygen decreased to close to null in all treatments (Figure 5.4). This sudden demand in oxygen was caused by aerobic organisms and is the reason why compost extracts must be provided with a high rate of oxygen (Ingham, 2002). At day 8, an increase in NH_4 was measured, up to 145.7 and 187.8 mg/L in the Manure & 1% Molasses and Manure & 1.5% Molasses respectively. Addition of molasses increased the available carbon source and promoted the growth of denitrifying bacteria (Jamieson et al., 2003). Anaerobic environment inhibit nitrification and low initial NO_3 content might have inhibited N_2 or N_2O formation (Jamieson et al., 2003; Quan et al., 2005). This was not observed in the control treatment, where nitrifying bacteria probably increased over time, and continuous decrease of NH_4 levels were measured (Figure 5.1). Therefore, when molasses is added to promote mineralization aeration should be maintained or even increased to promote NH_4 oxidation.

Nitrate content in this treatment doubled over the whole experiment, especially in the Manure & 1.5% Molasses treatment, where the concentration went from 1.1 to 2.2 mg/L (Figure 5.3). Daly and Stewart (1999) observed that an increase in mineralization of C resulted in the increase in NO_3, sulphate and phosphate concentration in soils as the microbial population are immobilizing nutrients (Daly & Stewart, 1999). The addition of molasses therefore promoted mineralization and increased nutrient availability for plant roots. Methanol is an alternative source of C that could be further investigated to determine if similar results could be achieved (Quan et al., 2005). Inoculation can also be explored to promote EM growth. Daly & Stewart (1999) recommended the use of additional compost and EM to promote nutrient mineralization and plant uptake of nutrients from compost (Daly & Stewart, 1999). However, use of C source should be monitored as unused carbon increases chemical oxygen demand and can cause effluent if disposed as municipal waste (Quan et al., 2005).

5.4.1 ISEs measurements

NH_4 measurements with ISE were varying from 15 to 28%, about 20% below the Lachat readings without adjustments (Figure 5.2). Adjusting the ISE readings with a linear regression model reduced the difference to 9%, varying from 2-16% above Lachat readings. For nitrate, if the values reach above 10 ppm, then it could be appropriate to use ISE. Large amount of variation within the same sample and in different treatments could have an impact on the ISE measurements

(Kim et al., 2007). A better understanding of the manure specific ionic strength and capacities can improve the measurements by the ISE electrode (Brouder et al., 2003).

5.4.2 Plant growth

A preliminary experiment was conducted to determine the effect of NH_4 on crop growth, where kale and lettuce were grown in a Hoagland and modified Hoagland solution in which $(NH_4)_2HPO_4$, KOH and $CaCl_2*2H_2O$ substituted $Ca(NO_3)_2*4H_2O$ and KNO_3 (Figure 5.5 and Figure 5.6). During the growth period, nutrient precipitation occurred, however nutrient solution was not analyzed, but will be explored in upcoming research. Kale and lettuce grown in the modified Hoagland solution were 6 and 4 times smaller than in the control treatment. Since the modified Hoagland solution was not deficient in other nutrients, the treatment effect observed was most likely caused by high NH_4 concentrations. Plants susceptible to NH_4 toxicity are lacking the ability to exclude NH_4 through the plasma-membrane influx system and accumulate excessive amounts of NH_4 in the cytosol (Goyal & Huffaker, 1984). These plants tend to increase their total root respiration imposed by the plasma membrane on the plant root system and result in a growth decline (Goyal & Huffaker, 1984). Even if the plants were smaller, they did not show sign of toxicity. In a greenhouse environment 40% of total nitrogen in ammonia-plus-urea form nitrogen is considered ideal for plant growth (Adams, 1997; Nelson, 1991). If the manure extract with molasses will be used for plant growth, extra NO_3 should be added and the nitrogen source needs to be balanced to prevent toxicity.

5.5 **Conclusion**

In a closed hydroponic system, use of EM with molasses and aeration stimulated nitrate production by two times and reduced ammonium by more than half in the chicken manure extract. EM with molasses increase C mineralization and release of nutrient in the solution, which would make nutrient uptake by plants roots easier. High NH_4 content can create a toxic environment for plant growth and NO_3 production or addition is required. Monitoring of manure extract maturity could be tracked by the dissolved oxygen levels and the decrease in NH_4 content over time with an ion-selective electrode.

5.6 Acknowledgement

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Connecting Text

In Chapter 5, the reduction of ammonium in a manure-extract solution was achieved with aeration in 12 days. Molasses was added in the solution to increase the carbon content and the beneficial microorganisms' activity. Nitrate content doubled, and ammonium content was halved during the experiment. Use of ion-selective electrode allowed to collect real-time data for ammonium.

Ammonium and nitrate require to be measured in a manure-based solution to prevent toxicity or deficiency. Different methods are made available to measure these nutrients in a water solution. In Chapter 6, the focus is on determining the optimal method for nitrate and ammonium monitoring in a manure extract solution. Three methods were compared: Lachat flame spectrometry, API water test kit, and ion-selective electrodes. Lachat flame spectrometry is the most commonly used methods, API water test kit is usually used in clear water, and ion-selective electrodes are becoming more accessible and have been used in wastewater analysis.

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6 NO_3 AND NH_4 MEASUREMENT IN HYDROPONIC SYSTEMS USING MANURE EXTRACTS SOLUTIONS WITH ISES, LACHAT FLOW INJECTION AND AQUARIUM WATER TEST KIT: A PILOT STUDY

Abstract

Manure-based extract solutions can substitute inorganic fertilizers in hydroponic systems. Plant nutrient uptake and the source of manure will influence the nitrate (NO_3) and ammonium (NH_4) content in the solutions. In this study, manure extracts were prepared from cow, chicken and turkey manure, and three methods were compared to measure their NO_3 and NH_4 content. API water test kit and ion-selective electrodes (ISEs) were compared to values recorded by a Lachat flow injection instrument. Before the API water test kit analysis, two pre-treatment steps were investigated to clarify the samples. ISE was the most reliable method to measure NH_4. The API test kit was not reliable even with the pre-treatment steps. NO_3 in the manure extracts was low and difficult to measure with the API and the ISE methods.

Keywords: hydroponic systems, ion-selective electrodes, organic fertilizer

6.1 **Introduction**

Hydroponic systems are soilless methods used for plant production and are often found in controlled environments such as greenhouses. These systems result in increased yield and minimize the use of water and chemical pesticides (Vardar et al., 2015). Compost teas have been investigated as a replacement to inorganic fertilizer in hydroponic systems (Carballo et al., 2009; USDA, 2013). The most common method to monitor nutrient levels in hydroponic systems is with a pH probe and an EC meter. The pH and EC sensors do not provide information on the concentration of individual ions, which makes it difficult to balance the nutrient solution as they are depleted over time (Kim et al., 2013).

In a solution made from manure, NH_4 needs to be monitored as it can be toxic for plants at high concentrations (Adams, 1999). NO_3 is the preferred form of nutrient for plants (Shinohara et al., 2011). Therefore, both NH_4 and NO_3 concentration should be monitored in a hydroponic system. Flow injection analysis is the commonly used method to monitor NH_4 and NO_3 in wastewater and hydroponic systems, however, this method can be costly and time-consuming (Kim et al., 2007; Parks & Milham, 2015). Alternatively, ion-selective electrodes have been used to monitor nutrient content in salt water and wastewater (Kim et al., 2013; Thomas & Booth, 1973), and API freshwater master test kit is used to measure nutrient content in aquariums.

The objective of this study was to compare three methods to measure NH_4 and NO_3 content in manure-based extracts (chicken, cow and turkey) used in hydroponic systems for lettuce and kale production. Measurements with a Lachat flow injection autoanalyzer, ion-selective electrodes and a commercially accessible API water test kit were compared to determine their accuracy and precision, and to find an inexpensive method that can provide the grower necessary information on the nitrate and ammonium levels in the solution.

6.2 **Materials and methods**

6.2.1 Preparation of manure-derived hydroponic nutrient solutions

Cow and chicken manure were sourced from McGill University's Macdonald Campus Farm in Sainte-Anne-de-Bellevue, Quebec, Canada. Turkey manure was sourced from Aviculture KDEM, Inc. (Saint-Gabriel-de-Valcartier, QC, Canada), where it was left outside for storage purposes and exposed to the external environment for 1–6 months prior to use. For each manure type, three aerated manure extract solutions were prepared with different concentrations as described previously (El-Shinawy et al., 1999; Leudtke, 2010). In brief, 20 g, 50 g and 100 g manure were separately diluted in 30 L tap water. Solutions were aerated with an air pump for 48 h then filtered through a 1-mm sieve to remove large fragments. Next, the solutions were diluted with tap water to reach a final volume of 60 L with concentrations of 10 g/L, 25 g/L and 50 g/L. Solutions were replaced every 14 days with freshly prepared solutions.

Six ebb and flow (recirculating) hydroponic systems were used, and each bed was flooded for 15 min every hour, resulting in a water depth of no more than 5 cm per bed. One bed contained modified full-strength Hoagland solution (Hoagland & Arnon, 1950) as a control. The other beds contained the chicken, cow, and turkey manure extract solutions at three different concentrations (10 g/L, 25 g/L and 50 g/L). Three replicas per plant (lettuce and kale) were grown for each manure source and each concentration.

6.2.2 Common traditional analytical procedures

Preliminary step

Three 50 mL samples of each manure extract solution were taken on a weekly basis. Samples were filtered with a Whatman qualitative filter paper No 1 (GE Healthcare Life Sciences, United Kingdom), and stored in a freezer at -78°C awaiting analysis. Samples were diluted 100 times before analysis.

Ammonium and nitrate measurement in solution

The ammonium concentration of each manure extract solution was determined using the Quickchem® method for ammonium, as per the manufacturer's instructions (Lachat Instruments, Milwaukee, WI, US). Briefly, equal amounts of the buffer solution and the sample was mixed. The buffer solution contained phosphate, salicylate and hypochlorite. The chemical reaction was amplified by heating the solution to 60°C. Ammonium was quantified by measuring the resulting green color at 660 nm with a flow injection autoanalyzer, according to the manufacturer's instructions (Lachat Instruments).

The Quickchem method (Lachat Instruments) was used to determine the nitrate concentration of each solution. A total of 5 ml for each sample was passed through a copperized cadmium column (Cu-Cd column) that reduced nitrates into nitrites (Otsuki 1978). When combined with a sulfanilamide and phosphoric acid reagent, nitrites turned magenta and this color was quantified at 520 nm with a flow injection analyzer, according to the manufacturer's instructions (Lachat Instruments).

6.2.3 Ion-selective electrodes

ISE calibration & measurement

Direct potentiometry was selected over one point calibration, incremental techniques and multiple sample addition, as this method of analysis is the most useful when the samples have a wide range of concentration (Toledo, n.d.). Two plastic membrane sensor with a double chamber reference system ISEs were used: detectION ® 3021 Nitrate and detectION 3051 Ammonium Combination (Nicosensors, Huntingdon Valley, PA, USA).

Calibration curves were calculated for NO_3 and NH_4 ISEs. For both electrodes, the electrode potentials developed were measured using a known stock solution of 10, 100 and 1000 ppm. The stock solutions were prepared with KNO_3 for NO_3 and NH_4Cl for NH_4 (Toledo, n.d.). For each reading, the tip of the ISEs was placed in 5 mL of sample or stock solutions. Ionic strength adjustment buffer (ISAB) were added to the stock solution and samples before measurement. For samples where NH_4 was measured, 1 M $MgSO_4$ was added at 10% v/v, as for NO_3, 2 M $(NH_4)_2SO_4$ was added at 2 mL per 100 mL of sample (Mettler Toledo, n.d.). To create a proper calibration curve, the ISEs were placed in the stock solution with a known quantity from the least concentrated solution to the most concentrated solution for 60 seconds and collected 4 measurements. Between each standard sample, the electrodes were rinsed with distilled water. Once the calibration measurements were taken, the unknown samples from manure extracts were measured, where the sensors were placed in the unknown sample for 15 seconds and rinsed between each sample.

6.2.4 API water test kit

Preliminary steps

To minimize the coloration of the samples and to remove fragments, two pre-treatment steps were tested: *i)* The manure extracts were filtered (Whatman #1) and *ii)* filtered, then centrifuged at 2,500 g for 5 minutes.

Sample Preparation

For the NO_3 measurement, 20 uL of solution #1 (labeled bottle provided in the API water test kit) was added to 200 uL of the manure extract (API® Aquarium Pharmaceuticals, Chalfont, PA, USA). The solution was shaken for a few seconds, then 20 uL of solution #2 (labeled bottle provided in the API water test kit) was added. The samples were shaken for one minute and left idle for five minutes for the color to develop. A total of 80 uL from the sample was used to measure with a PowerWave XS Universal Microplate spectrophotometer (BioTek Instruments Inc, Winooski, Vermont, USA).

For the NH_4 measurement, 16 uL of solution #1 and #2 (from the labeled bottle provided in the API water test kit) was added to 200 uL of the manure extract. The solutions were shaken for a few seconds before being left idle for five minutes. A total of 77 uL was used for measurement in the spectrophotometer (API® Aquarium Pharmaceuticals).

Following the API water test kit protocol for NH_4 and NO_3, the results were read by a PowerWave XS Universal Microplate spectrophotometer to minimize human error. Readings were recorded at 440, 480, 517, 562, 570 and 646 nm. If the readings were above the detection rate, the solutions were diluted and re-tested. In each plate, there was a reference solution used to determine the unknown solution concentration. Reference solutions for NH_4 were NH_4NO_3 + NH_4Cl, and $NaNO_3$ + NH_4NO_3 for NO_3. For each reading, a reference solution was determined with a linear and exponential equation, then compared to the manure extract solution measured by the Lachat flow injection instrument.

6.2.5 Statistical analysis

JMP version 13.2.1 (SAS, Cary, NC, USA) was used for ANOVA testing to determine the difference between the Lachat, ISE and API measurements. A regression model was used to adjust values from ISEs to the Lachat readings. A significance of $p < 0.05$ was employed for all statistical tests.

6.3 Results and discussion

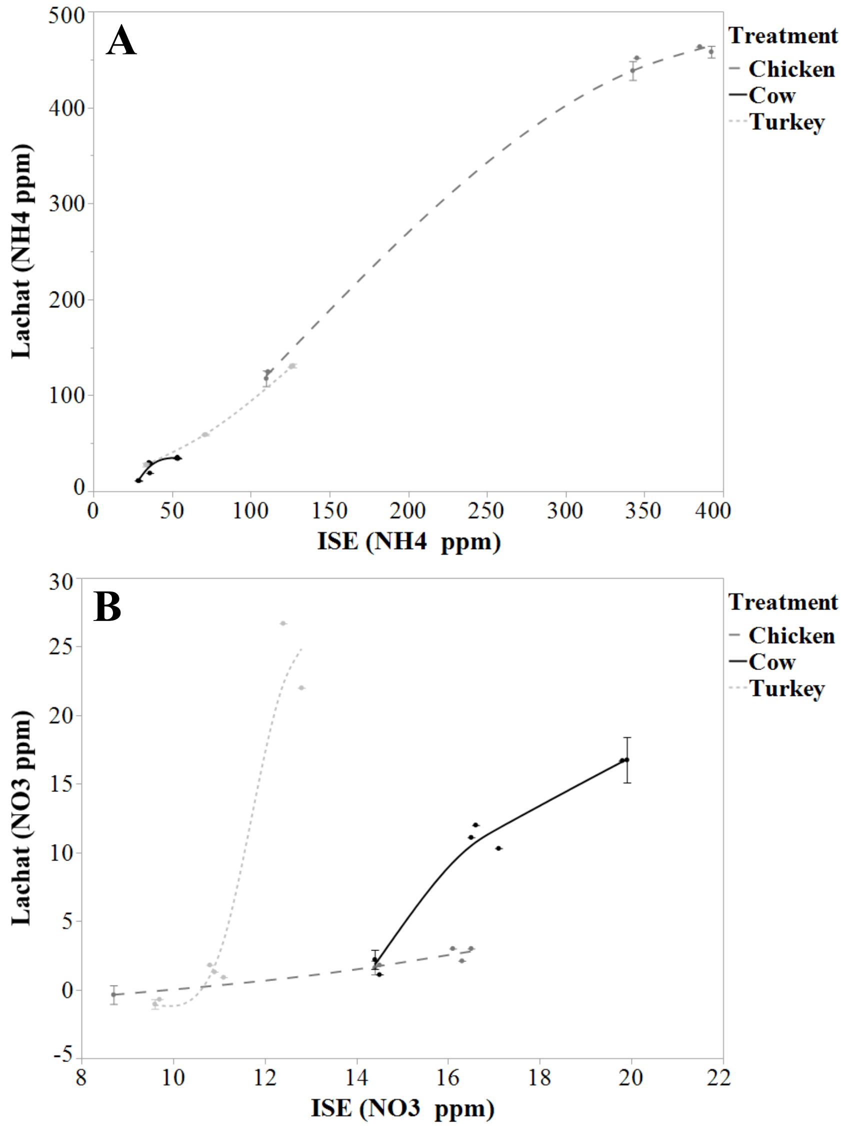

Figure 6.1. Regression fit between ISE and Lachat measurements with a second order polynomial equation to determine the content in manure extracts at different concentrations for A) NH_4, and B) NO_3.

Table 6.1 Second order polynomial equations with the highest R^2 for NO_3 and NH_4 determination using ISEs.

ISE	Treatment	Equation	R^2	RMSE	ANOVA Prob>F
NO_3	Chicken	$C_{NH4} = -4.6315 + 0.4341 * ISE_{NO_3} + 0.0246 * (ISE_{NO_3} - 13.1444)^2$	0.793	0.797	0.0089*
	Cow	$C_{NH4} = -36.1813 + 2.8276 * ISE_{NO_3} - 0.4054 * (ISE_{NO_3} - 17.0111)^2$	0.960	1.522	<0.0001*
	Turkey	$C_{NH4} = -82.7934 + 7.7828 * ISE_{NO_3} + 3.2601 * (ISE_{NO_3} - 11.0556)^2$	0.909	4.039	0.0025*
NH_4	Chicken	$C_{NH_4} = -4.6315 + 0.4341 * ISE_{NH_4} + 0.0246 * (ISE_{NH_4} - 13.1444)^2$	0.998	9.521	<0.0001*
	Cow	$C_{NH_4} = -15.1743 + 1.1752 * ISE_{NH_4} + 0.0642 * (ISE_{NH_4} - -39.2778)^2$	0.910	3.744	0.0007*
	Turkey	$C_{NH_4} = -17.6925 + 1.0781 * ISE_{NH_4} + 0.0049 * (ISE_{NH_4} - 77.0111)^2$	0.999	1.637	<0.0001*

API water test kit and ISEs measurements for NH_4 and NO_3 were statistically different than the Lachat flow instrument ($p < 0.05$). A calibration factor has been previously used to correct for the differences in ISE values (Kim et al., 2007). In this study, second order polynomial equations adjusted ISE readings for each manure solutions (Table 6.1). A linear fit model was also tested, but higher R^2 values were achieved with second order polynomial equations.

The highest concentration of NH_4 was recorded in chicken manure extract at 459.9 ppm followed by turkey and cow extract with 130.6 ppm and 34.8 ppm respectively (Figure 6.1 A). These readings were properly monitored by the NH_4 ISE after adjustment. Concentrations of NO_3 in the manure solutions were below 20 ppm (Figure 6.1 B), which was close to the detection limit for both ISE and API water test kit. The detection limit of the ISE is reported to be 5 ppm and 0 ppm for the API water test kit (API Aquarium Pharmaceuticals, 2009). After calibration for NO_3 with stock solutions, ISE readings were consistent for values above 15 ppm and above 20 ppm for the API. High accuracy is not necessary at low NO_3 concentration as low concentrations of NO_3 are not toxic for plants, however, NO_3 should be monitored at higher concentration to limit toxicity.

Use of additional pre-treatment steps for the API water test kit had a small effect on measurements. Since the API water test kit was a colorimetry method, coloration, turbidity, and presence of unknown ions at an unknown concentration in the sample would have impacted the reading (Pansu & Gautheyrou, 2006). The effect of turbidity was noticed on the chicken manure treatment where API values were greater than 1,000 ppm (Appendix, Table 6.2). Centrifuging the samples to minimize solid particles reduced the difference between the Lachat readings but were significantly different than the actual value from the Lachat readings (over 4,000% for chicken manure, 400% for cow manure and below 75% for turkey manure). The difference in chicken manure could have been caused by coloration or a reaction with the API solution during manipulation. For the cow and turkey samples, the closest results for NH_4 were with the paper filter pre-treatment and NH_4Cl at 517 nm. For NO_3, the closest measurements for cow samples were with NH_4NO_3 at 517 nm (Appendix, Table 6.3). For turkey and chicken, the values calculated were always 300% above the readings of the Lachat instrument.

ISEs provides real-time readings over API water test kit and Lachat readings. ISEs are more affordable than Lachat flow instrument and can be easily transported to sampling sites. All three methods require daily calibration (Toledo, n.d.). At a high NH_4 and NO_3 concentration, samples must be diluted before being analyzed by the API water test kit and the Lachat instrument, but this step was not required for the ISE as the NH_4 values are within the reading limits. To minimize human error a colorimeter apparatus is necessary for API readings. However, API water test kit is very sensitive to any discoloration and has limited ability to measure manure extract solutions.

Improved R^2 coefficient for ISE calibration can be achieved by increase the sampling size within treatments, calibrating ISEs using manure extracts with known nutrient content, and preparing stock solutions with concentrations closer to the manure extracts content. Stock solutions of NH_4 should range between 10-500 ppm and between 10-250 ppm for NO_3.

6.4 Conclusion

Real-time readings and quick calibration of ISEs offer an advantage over the Lachat and API methods. ISEs are more affordable and can be easily transported to sampling sites. Adjusted ISE NH_4 readings with a second order polynomial equation improved measurement accuracy. NO_3 content in the manure extracts were near the detection limit of the ISE (below 20 ppm). API measurements were affected by the coloration of the manure extracts. Even with the integration of pre-treatment steps to reduce turbidity of the samples, the API readings were over 300% different than the Lachat values.

6.5 Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), Discovery Grant 355743-13.

6.6 **Appendix**

Table 6.2. NH_4 measurement in manure extract.

Treatment	Measurement method			
	Lachat (ppm)	API water test kit (ppm)		ISE (ppm)
		NH_4NO_3	NH_4Cl	
Chicken 1	459.9 ± 4.0	1,651,995.2 ± 14,702.5	151,639.6 ± 2,219.8	389.9 ± 0.1
Chicken 2	442.9 ± 7.2	1,390,958.8 ± 26,470.7	235,846.3 ± 6,794.4	343.9 ± 0.0
Chicken 3	120.0 ± 5.3	--	--	110.6 ± 0.0
Cow 1	11.2 ± 0.0	11.4 ± 0.5	7.6 ± 0.2	28.7 ± 0.0
Cow 2	34.8 ± 0.4	272.0 ± 2.1	96.1 ± 1.4	53.4 ± 0.0
Cow 3	26.2 ± 3.5	--	--	35.6 ± 0.0
Turkey 1	130.6 ± 1.2	129.2 ± 2.5	112.2 ± 7.1	126.4 ± 0.0
Turkey 2	59.2 ± 0.5	67.8 ± 1.4	62.7 ± 3.3	71.0 ± 0.0
Turkey 3	27.8 ± 1.0	--	--	33.6 ± 0.0

Table 6.3. NO_3 measurement in manure extract.

Treatment	Measurement method Lachat (ppm)	API water test kit (ppm) NH_4NO_3	API water test kit (ppm) $NaNO_3$	ISE (ppm)
Chicken 1	1.6 ± 0.3	21.4 ± 0.7	20.3 ± 1.3	14.4 ± 0.0
Chicken 2	2.7 ± 0.3	15.4 ± 0.9	16.6 ± 2.2	16.3 ± 0.0
Chicken 3	0.4 ± 0.7	--	--	8.7 ± 0.0
Cow 1	16.8 ± 0.9	17.4 ± 0.4	15.8 ± 1.0	19.9 ± 0.0
Cow 2	11.2 ± 0.5	14.5 ± 0.5	15.1 ± 0.8	16.7 ± 0.0
Cow 3	1.9 ± 0.5	--	--	14.4 ± 0.0
Turkey 1	0.9 ± 0.2	14.5 ± 0.3	15.5 ± 1.1	9.7 ± 0.0
Turkey 2	1.3 ± 0.3	22.0 ± 0.7	22.1 ± 1.1	11.0 ± 0.0
Turkey 3	24.3 ± 0.5	--	--	12.7 ± 0.0

Table 6.3 shows values for manure only paper-filtered. For all treatment, there was a significant difference between the measurement method used to access the NO_3 content in the manure extract (Lachat, API with either NH_4NO_3 or $NaNO_3$ and ISE) ($p < 0.05$). Best readings for the API test kit was recorded at 517 nm.

Connecting Text

In Chapter 6, three methods were compared to determine the simplest way to monitor ammonium and nitrate in a manure extract solution. Colorimetry was not suitable as there will always be a discoloration even after the solution is filtered. Use of ion-selective electrodes is the simplest method and allows for continuous monitoring of the solution; however, calibration is required. Lachat flame spectrometry is the most accurate and precise method, yet it is time-consuming.

One of the advantages of an ebb-and-flow hydroponic system is that the irrigation can be controlled based on the plants need. In Chapter 7, a CAN bus system is developed to control the irrigation in different hydroponic beds. This system allows collecting moisture and temperature data from the hydroponic beds with different pre-set values to turn on and off the watering system. This approach is simple to set up for different crops without the necessity to reprogram the primary software used.

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7 DEVELOPMENT AND MONITORING OF WATER STRESS ON LETTUCE AND KALE GROWN IN HYDROPONIC SYSTEMS WITH A CAN BUS SYSTEM

Abstract

Controlled Area Network (CAN) bus systems designed for greenhouse monitoring have been proposed to measure soil moisture content, yet they are still absent from hydroponic systems. In this study, irrigation control, monitoring of substrate moisture levels and temperature were achieved using a CAN bus system connected to hydroponic beds. In total, five nodes were mounted on five hydroponic beds and two irrigation methods were compared on lettuce and kale: 1) a pre-set timer activated the irrigation pump, and 2) irrigation pumps were activated using predetermined moisture levels. A statistically significant effect ($p < 0.05$) on plant height, dry and wet mass was achieved after 29 days of growth. The highest yield was measured in the timed treatment, where moisture varied from 88%-100%, and lowest in the 25%-85% treatment. Individual plant wet and dry mass was monitored using a remote sensing instrument and confirmed at harvest by weighing. The remote sensing instrument consisted of a laser, ultrasonic and thermal infrared that followed a circular path and collected aerial crop information (height, leaf size, and near infrared).

Keywords: CAN bus system, hydroponic system, irrigation

7.1 Introduction

Hydroponic systems are commonly used to study plant response to biotic and abiotic stresses (Both et al., 1997). Hydroponic systems are easily implemented, offer better environmental control and are easier to monitor than experiments conducted in agricultural fields. Irrigation timing and nutrients concentration are some of the numerous features that can be modified in hydroponic systems to optimise yield or to determine the impact of varying environmental parameters on a plant. The most common irrigation method in greenhouses relies on pre-set timers to water plants, however, it is uncertain if this approach is optimal for plant growth.

Recently, various CAN bus systems have been designed and implemented in greenhouses (Li & Zhang, 2010; Lihong et al., 2011; Liu et al., 2007; Pengzhan & Baifen, 2010; Zhang et al., 2009). Unfortunately, there is no literature using such systems for hydroponic systems. CAN bus designs are broken down into nodes, that record the data, and a central PC that collects the data from the nodes (Wang et al., 2009). Sensors are connected to a node which can be microprocessors or microcontrollers that stores the data and transmit the information through the bus system. Using a CAN bus system simplifies the control of the greenhouse environment, especially covering large areas. In addition, it minimizes the response time compared to analog systems (Pengzhan & Baifen, 2010). CAN bus systems can allow for the collection of real-time data, such as temperature, light and soil moisture (Liu et al., 2007). The data collected can then be used to optimise yield and crop quality (Liu et al., 2007).

During an experiment, to determine a treatment effect on crop biomass, aboveground wet and dry mass is usually measured by a destructive method, where plants are removed from the treatment. This approach necessitated the increase in the sample size especially if the samples are collected over a long period (Both et al., 1997; Thompson et al., 1998). Therefore, non-destructive methods are more desirable. Such methods include the following: biomass estimation using remote sensing cameras and leaf area index measurements (Asner et al., 2003; Hunt et al., 2005).

The objective of this research was to develop a CAN bus system with independent nodes that would monitor temperature and moisture content of hydroponic beds, and a central PC that would collect information from the nodes. The measurements collected from the beds would be used to activate and turn off water pumps in ebb and flow hydroponic systems. Different moisture levels were compared to a bed with timed irrigation to assess if water stress occurred during growth of lettuce and kale. A remote-sensing instrument was used to assess crop biomass using plant height information.

7.2 Materials and methods

7.2.1 Plant culture

The experiment was conducted in a greenhouse located at Macdonald campus of McGill University, Canada (45.4079° N, 73.9388° W) between March and May 2017. Seeds of red Russian kale (*Brassica napus* 'Red Russian') (S8422-001, Richters, Goodwood, ON, Canada) and romaine lettuce (*Lactuca sativa* var. longifolia) (Ridgeline Pelleted MT0 OG, Johnny's, Winslow ME, USA) were germinated in pre-washed rockwool rooting medium cubes (Grodan, Roedmond, NL). For each species, 12 cubes were seeded with two seeds and then transferred to a tray. The tray was moved into a growth chamber for two weeks at a constant temperature of 25ºC, 50% humidity and 16:8 h of day: night. Distilled water was added when necessary to keep the rockwool wet. One week after germination, the seedlings were thinned to one seedling per rockwool cube. The seedlings were grown for two weeks in a growth chamber. The plants were placed in 4-inch rockwool cubes and transferred to an ebb and flow hydroponic system in a greenhouse. A 60 L full-strength Hoagland solution was replaced every 14 days for each treatment (Hoagland & Arnon, 1950). Solutions were aerated with an aquarium air pump (Marina Air Pump 200, Rolf C. Hagen (UK) Ltd., West Yorkshire, UK). Within the hydroponic beds, the seedlings were placed randomly to minimize the edge effect.

7.2.2 CAN bus system setup

The CAN bus system consisted of a PC host that worked as the main monitoring system, the CAN bus, and a node attached to each hydroponic bed, which collected the temperature and moisture information (Figure 7.1). The CAN bus design consisted of five independent nodes using

Arduino UNO (Atmega328P Board) that were connected between one another with a CAN bus shield (Jaycon systems Can Bus Shield v1.1) mounted on five hydroponic beds. Each Arduino UNO controlled the hydroponic system's water pump (Aqua One, Aqua Pacific UK Ltd, Romsey, UK) and was connected to a moisture sensor (SEN0114 Gravity: analog soil moisture sensor) and a thermometer (#81-ADA Waterproof DS18B20). A separate Arduino UNO with a CAN Bus shield was relayed to a JBC311U93 computing system (Intel Atom, 2GB RAM, 32GB SSD from Jetway Computer Corporation, Newark, CA, USA). This computing system was reading and storing the information sent by each node on the CAN Bus shields. The computing system had its own LAN network that was used to upload data to a cloud where the end user had access to the data anywhere through a website. Sensors were placed at least two inches apart in two separate rockwool cubes to avoid any interference. Treatment and node control was set based on minimum and maximum moisture content (Table 7.1). Temperature sensors were place in individual rockwool blocks.

All plants were harvested 29 days after the start of the treatment. Plant were measured for aboveground wet and dry mass, maximum height, maximum extended height (length of the plant harvested, placed on a flat surface and measured), and the number of leaves (leaves greater than 2 x 2 cm). The plants were dried at 40^{o}C for a week and were weighted, grounded, and stored in plastic containers.

Table 7.1 Moisture content set-up to activate the water pumps in the ebb and flow hydroponic systems.

Node ID	Min moisture	Max moisture
1	50%	85%
2	50%	100%
3	25%	85%
4	50%	75%
5 (Control)	Automated system, water pump active 15 min every hour.	

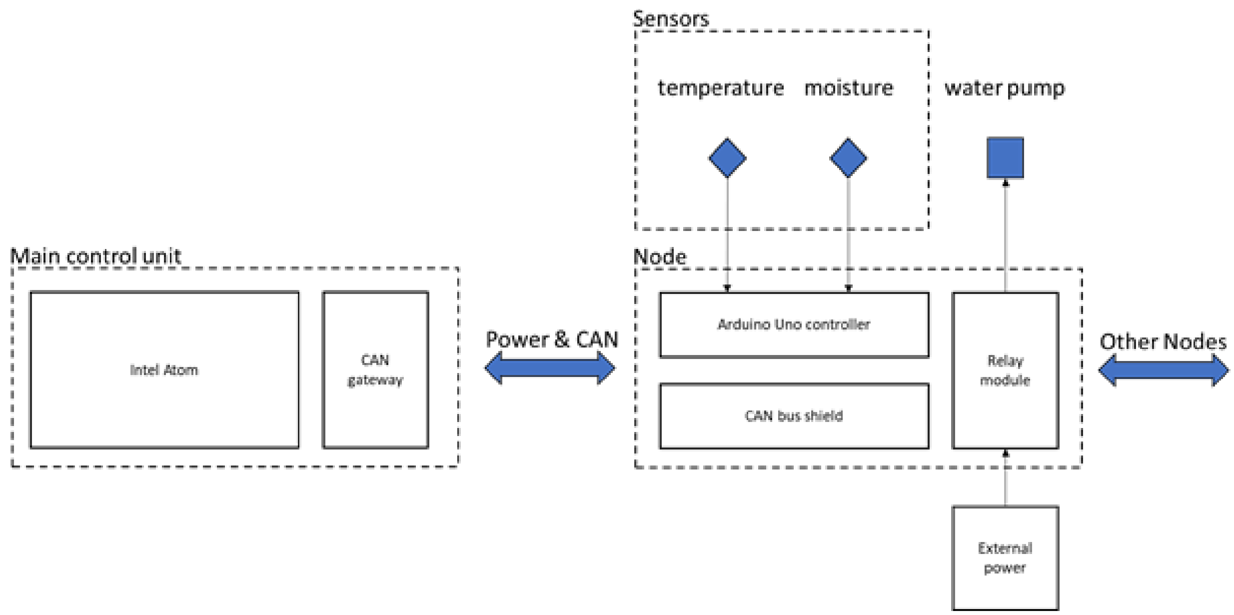


Figure 7.1 CAN bus system setup

Instrument prototype for crop biomass estimation

A crop canopy instrument was developed to determine the aboveground wet and dry mass using laser and ultrasonic measurements (Buelvas & Adamchuk, 2017). The IL-600 laser (Keyence, Itasca, IL, USA) and the ToughSonic14 ultrasonic sensor (Senix, Hinesburg, VT, USA) provided plant height measurements. The sensors were mounted on a 3D-printed holder that was connected to a controlled stepper motor (T-NM17C04) (Zaber Technologies, Vancouver, BC, Canada) to allow for a circular movement at a specific radius (Buelvas & Adamchuk, 2017). The laser sensor identified canopy structures, while the ultrasonic sensor identified the space within and around the plants.

The prototype was used to measure physiological characteristics of the plants before they were harvested. Each sensor provided a measurement every 45 ms, where the motor was set at a constant speed of 20^{o} s^{-1}, providing a data point every 0.9^{o} (Buelvas & Adamchuk, 2017). The instrument was placed within the hydroponic bed above both kale and lettuce and 5 rotational cycles were recorded at three locations within the hydroponic bed. Aboveground measurements were collected 18, 22, 25 and 29 days after start of treatment.

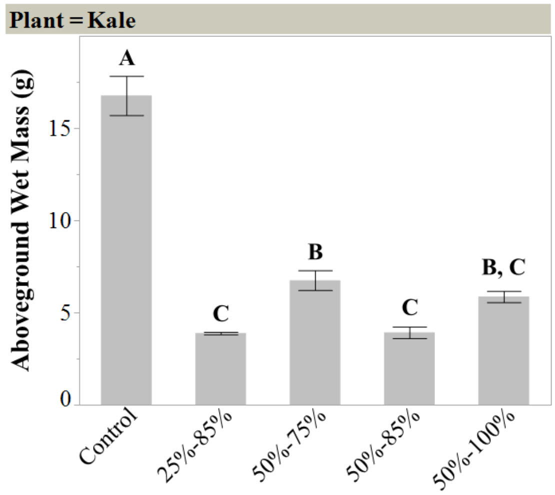
Statistical analysis

A completely randomized design with temporal repeated measurements statistical test was used to optimize the model for the prototype biomass estimation. Analysis of variance (ANOVA) was conducted to determine the difference among each treatment. Post-hoc comparisons were accomplished using Tukey's honest significance test (HSD) using JMP 13.2.1 (SAS, Cary, NC, USA) on the results to determine difference between treatments ($p < 0.05$).

7.3 Results and discussion

7.3.1 Effect on growth

Moisture levels used to activate the water pumps had an impact on plants yield. For both lettuce and kale, the aboveground wet mass was significantly ($p < 0.05$) higher for the control treatment where the irrigation was turned on with a timer for 15 min every hour (Figure 7.2). The average wet mass in the control treatment was 42.2 ± 4.9 g for lettuce and 16.8 ± 1.1 g for kale. Moisture content in the control treatment was fluctuating between 88%, prior the activation of the pumps, to 100%, when the bed was flooded. Lowest yield was in the 25%-85% treatment with 3.9 ± 0.1 g for kale and 4.6 ± 0.6 g for lettuce. These results showed that crops have similar irrigation requirements and that a high moisture environment is preferred. Karam et al. (2002) conducted similar field research and showed that water stress has a significant effect on the mass of lettuce (Karam et al., 2002).



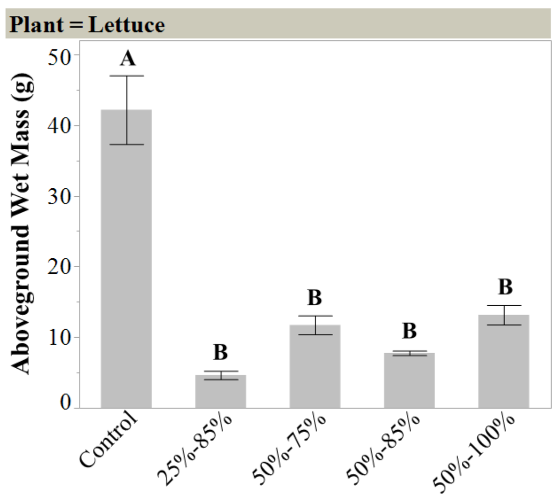


Figure 7.2 Aboveground wet mass for kale (*Brassica napus*) and lettuce (*Lactuca sativa* var. longifolia) for each treatment (with standard error). Letters above the columns show HSD statistical significance ($p < 0.05$).

A significant difference was measured for the aboveground dry mass for both plants ($p < 0.05$). The control treatment had the highest dry mass with 1.2 ± 0.1 g and 2.4 ± 0.3 for kale and lettuce respectively (Figure 7.3). Karam et al. (2002) found a reduction of 30 to 38% in dry mass of lettuce created in a water stressed environment. In this experiment, lowest yield was recorded in the 25%-85% and 50%-85% treatment for kale with 0.4 ± 0.0 g, while it was in the 25%-85%

for lettuce with 0.4 ± 0.0 g. This was a dry mass reduction of 80% for lettuce and 67% for kale compared to the control treatment. Similar measurements were recorded for lettuce when the irrigation started at 50%-85% (with 0.5 ± 0.0 g). This was not the case for kale, where the dry mass was fluctuating in the different treatments. The experiment conducted by Karam et al. (2002) lasted for 70 days and 66 days for Acar et al. (2008) (Acar et al., 2008). Elongation of the experiment for kale could determine if the different irrigation treatments would stay the same or converge over time, like lettuce.

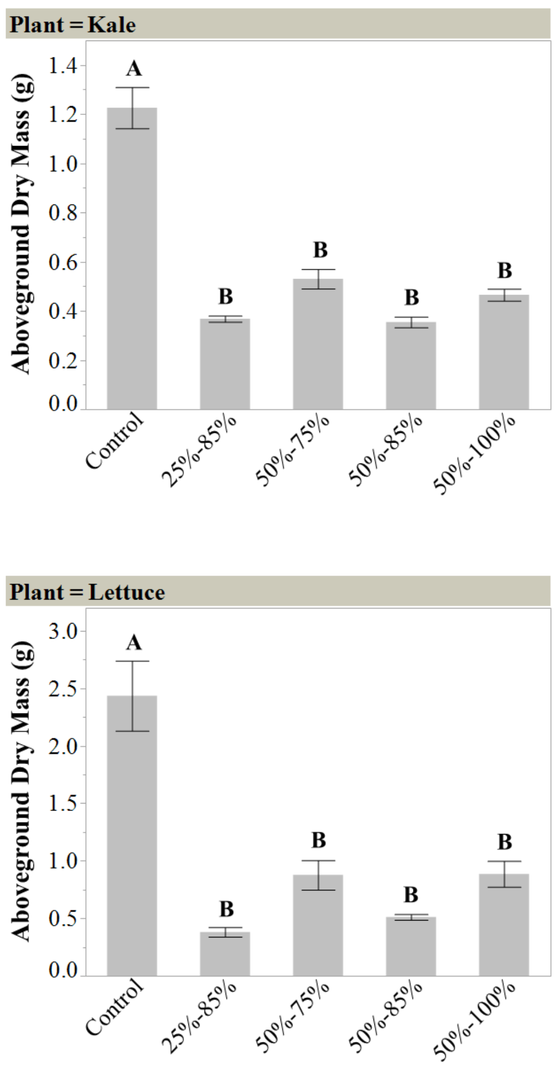


Figure 7.3 Aboveground dry mass for kale (*Brassica napus*) and lettuce (*Lactuca sativa* var. longifolia) for each treatment (with standard error). Letters above the columns show HSD statistical significance ($p < 0.05$).

The effect of water deficit was noted by the reduction of leaf numbers ($p < 0.05$), and height of the plants. Lettuce grown in the control and 50%-100% treatment had more than 12 leaves, while it had a minimum of 9 leaves in the 25%-85% treatment, a reduction of 25%. Maximum number of leaves for kale was in the control treatment as well with over 10 leaves, and lowest in the 50%-85% treatment with 8 leaves, a reduction of 20%. The effect on lettuce was noticed by Karam et al. (2002), where water-stressed treatments had 8-14% lower mature leaves (Karam et al., 2002). Irrigation treatment had a direct effect on height for both crops ($p < 0.05$). Highest extended height was recorded for both plants in the control treatment with 28.3 ± 0.4 cm and 26.3 ± 0.4 cm, and lowest in the 25%-85% treatment with 15.4 ± 0.4 cm and 14.9 ± 0.5 cm for kale and lettuce respectively (Figure 7.4). These results were similar to the wet mass, where the highest values were recorded for control and lowest for the 25%-85% treatment. Acar et al. (2008) recorded the tallest plants in the highest irrigated treatment. Growing plants in an environment lacking water stress, such as a nutrient film technique or in a continuously flooded bed could increase yield more than the ebb and flow system.

The experimental design used in this study impacted the water stress created. In an ebb and flow system, water submerges the rockwool from below ground level. In Karam et al. (2002) and Acar et al. (2008) experiment, a drip irrigation was used, where the plants were directly watered from the surface, reaching the roots directly. In this study, plants in treatments with a maximum of 75% and 85% humidity could have reduced water accessibility to roots, especially the first week after transplant, when the roots were the shortest and could have induced further water stress.

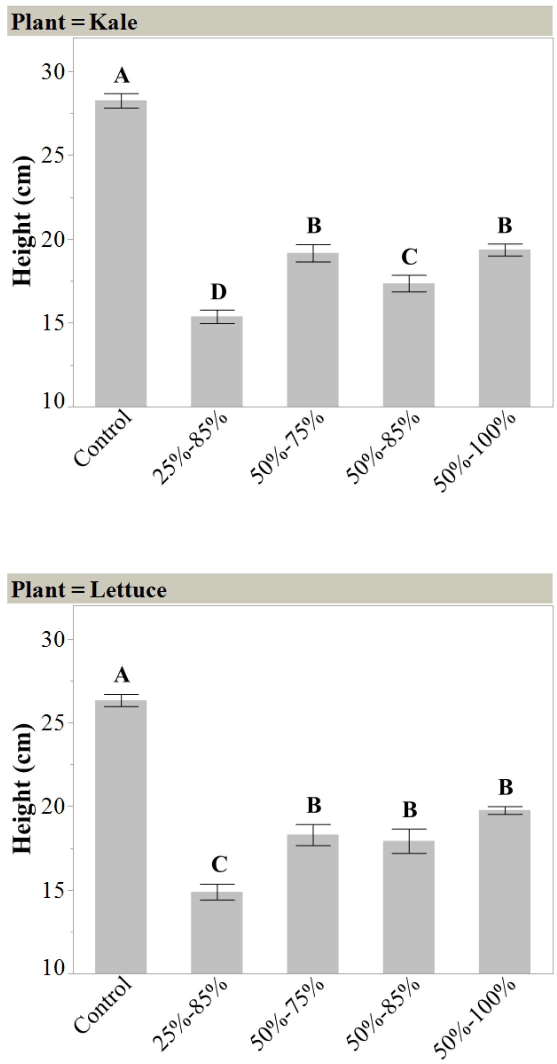


Figure 7.4 Aboveground height for kale (*Brassica napus*) and lettuce (*Lactuca sativa* var. longifolia) for each treatment (with standard error). Letters above the columns show HSD statistical significance ($p < 0.05$).

Table 7.2. Regression equation for crop response

Plant	Plant characteristic	Equation	R^2	RMSE	ANOVA Prob>F
Kale	Height	$Height = 12.0801 + 13.2128 * Irrigation_{start}$	0.311	1.947	<0.0001*
	Aboveground wet mass	$AWM = 2.207 + 6.705 * Irrigation_{start}$	0.119	1.810	0.0053*
	Aboveground dry mass	$ADM = 0.2836 + 0.3381 * Irrigation_{start}$	0.064	0.128	0.0439*
Lettuce	Height	$Height = 5.6296 + 14.934 * Irrigation_{start} + 6.4983 * Irrigation_{end}$	0.466	1.973	<0.0001*
	Aboveground wet mass	$AWM = -2.016 + 26.6226 * Irrigation_{start}$	0.274	4.736	<0.0001*
	Aboveground dry mass	$ADM = 2.207 + 6.705 * Irrigation_{start}$	0.164	0.400	0.0012*

Regression models were tested to determine plant characteristics based on initial ($Irriation_{start}$) and end of irrigation ($Irrigation_{end}$) moisture values (Table 7.2). For plant height, best result was for lettuce with a $R^2 = 0.466$, while it was only $R^2 = 0.311$ for kale ($p < 0.0001$). End of irrigation did not have a significant effect on kale height and was removed from the model. For aboveground wet and dry mass, the irrigation ending time was not significant and was removed from the model. For both kale and lettuce, even if the ANOVA test were significant (marked by *), the R^2 values were below 0.3.

7.3.2 Substrate effect on yield

Use of rockwool might have created a water stress effect by itself. Acar et al. (2008) conducted an irrigation experiment within a greenhouse, without the use of hydroponic systems, and found no effect of irrigation levels on head mass, number of leaves, or height of plants (Acar et al., 2008). Since the soil depth was greater than 80 cm, it might have retained more water than the substrate used in hydroponic systems. Rockwool is known to have a very restricted root environment and a lower buffering capacity for water and nutrients (da Silva et al., 1998). Suction created by lettuce and kale could have reduced the water available for uptake, even with an elevated water moisture measurement (da Silva et al., 1998). Acar et al. (2008) proposed a better water utilization by the crop as a result of good soil-water-air relationship with higher oxygen concentration in the root zone and a higher water content (da Silva et al., 1998).

7.3.3 CAN bus system recommendations

The CAN bus system developed in this study to monitor temperature, moisture levels, and to activate water pumps, could be implemented in greenhouses to monitor the environment with multiple hydroponic systems growing a wide variety of crops. The main advantage of this system is its versatility as nodes and sensors could be added without significant modification. Each node could also be reprogrammed based on the crop's requirements. Current and supplementary sensors (such as light sensor) would allow to collect data on a specific crop during growth and to improve its irrigation, yield and quality. Similar designs were developed for greenhouse monitoring but were lacking irrigation control and were not tested on crops (Lihong et al., 2011; Pengzhan & Baifen, 2010). A wireless sensor network prototype, already assembled and tested, would be a valuable add-on real time remote monitoring and minimize physical connections (Liu et al., 2007).

7.4 Conclusion

Moisture and temperature monitoring of hydroponic beds with a CAN bus system could be used to collect real-time data on the growing environment of a specific crop. In this study, the CAN bus system was used to induce water stress on both lettuce and kale using different moisture levels to start and turn off pumps. Highest yield was measured in the control treatment, where the moisture was between 88%-100%. Lowest yield was recorded in the 25%-85% treatment. Activation of irrigation pumps at higher moisture levels would promote yield levels closer to the control treatment. In addition, substrate used in hydroponic systems will also have an impact on the water retention and the water stress created on plants.

7.5 Acknowledgement

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), Discovery Grant 355743-13.

Connecting Text

In Chapter 7, a CAN bus system was used to record data on the moisture and temperature levels of rockwools in hydroponic systems, then activate and turn off water pumps based on pre-set moisture levels. Lettuce and kale were grown in different hydroponic beds. The results showed that the highest yield was achieved in the control treatment, where the highest moisture levels, between 88%-100%, were maintained and the lowest was in the 25%-85% treatment. In general, CAN bus systems allows for an easier setup for greenhouses growing different plants with individual irrigation requirements.

PoultryPonics facility is a controlled environment located in Hay River, Northwest Territories of Canada. The facility was set up to raise chickens and to grow plants with hydroponic systems. In Chapter 8, a nutrient sensor is developed to be implemented in the facility with a biodigester. These add-ons would allow to use the chicken manure and to use it as a fertilizer for the hydroponic system. The biodigester would promote nitrification, and the nutrient sensor uses ion-selective electrodes to monitor the nutrient levels. Once operational, this facility would allow remote northern communities to grow their food, reduce the cost of food and address the existent food insecurity.

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8 DESIGN OF A BIOREACTOR AND NUTRIENT MONITORING INSTRUMENT FOR FOOD PRODUCTION IN HYDROPONIC SYSTEMS LOCATED IN NORTHERN CANADA

Abstract

Northern food productions are constrained by cold climates and a short growing season. Daily, northern food security affects over 300,000 Canadians. To address this issue and grow their own food, Polar Eggs, a poultry facility located in Hay River, Northwest Territories, Canada, developed the PoultryPonics facility, a controlled environment where both chicken and vegetables, such as kale and lettuce, can be grown year-round. To optimize the facility and reduce the inputs and waste produced, a chicken manure based fertilizer was developed as an alternative to inorganic fertilizers. This research involved the design, construction, and field-testing of a nutrient measuring system for K, NO_3, NH_4 and Ca using ion-selective electrodes (ISEs) linked to a bioreactor within the PoultryPonics facility. The bioreactor was used to aerate the manure, to promote nitrification, and mix other organic residues to balance out the nutrient solution. The bioreactor had two filters to reduce transmission of small particles and the manure solution was transferred to a heated bucket to minimize the risk of pathogen contamination. When the solution was ready, it was sampled and adjusted to a balanced plant nutrient level. Next steps include the balancing of the manure solution to meet different plants nutrient requirements and the calibration of the ISEs to reduce drift occurring over time.

Keywords: bioreactor, organic fertilizer, controlled environment, ion-selective electrodes

8.1 Introduction

Food insecurity is prevalent in northern Canada, especially in the Northwest Territories and Nunavut. Transportation costs, stores operating costs, food spoilage and inventory costs are some of the main factors that increases food price (Government of Canada, 2014). Weekly food budgets in northern regions cost over double the costs of other cities in Canada, $209 CAD in Ottawa compared to $328 to $488 in isolated Inuit communities (Government of Canada, 2015). In addition, 46.8% and 24.1% of total households in Nunavut and the Northwest Territories respectively are food insecure (Statistics Canada, 2012). In other provinces of Canada, these numbers are between 10-15% (Statistics Canada, 2012). To resolve this issue the Canadian government has subsidized food through the Nutrition North Program (Government of Canada, 2015).

Polar Eggs are a poultry facility in Hay River, Northwest Territories. They have developed the PoultryPonics system, a controlled environment facility, where chickens are raised, eggs are produced, and vegetables are grown. The PoultryPonics facility allows for year-round food production, independent of the northern climate. This project was developed to impact local economy of northern communities by providing a technology to allow community-based food production and reduce dependence on food importation. To minimize production costs, a project was put forward focusing on recovering chicken manure in hydroponic systems that could be independent of inorganic fertilizers.

Hydroponic plant production is a soilless agricultural practice which uses a nutrient solution and an artificial rooting medium to support plant growth (Kumar & Cho, 2014). These systems are mostly employed in greenhouses where the environment can be controlled and production can occur year round (Lee & Lee, 2015). Hydroponic systems typically use less water and have higher water efficiency, as the nutrient solutions are not lost through ground infiltration (Surendran et al., 2016). Productivity per unit area of a hydroponic system is higher than field grown agriculture and growing in controlled environments allow to cultivate plants with higher and more uniform nutritional values (Surendran et al., 2016; Suvo et al., 2017).

Compost teas have been investigated in agricultural production as a replacement or supplement to mineral fertilizer (Carballo et al., 2009). Compost teas, a type of compost extract, are watery extracts prepared with compost or compost mixed with manure. Compost teas are rich in effective microbes (EM) that are known to improve yield and quality of vegetable crops (Daly & Stewart, 1999). Manure is also a known source of EMs and could reduce the cost of compost tea production (Ch'ng et al., 2013).

The most common method to monitor nutrient levels in hydroponic systems are with a pH probe and an EC meter. However, these instruments do not provide information on the concentration of individual ions, which makes it difficult to balance the nutrient solution as they are depleted over time (Kim et al., 2013). Ion-selective electrodes are sensors with an ion-selective membrane that allows for the measurement of one analyte in a solution (Kim et al., 2013). Three ISEs are commonly used in hydroponic systems: NO_3, K and Ca (Kim et al., 2013; Kim, Shim, et al., 2015; Vardar et al., 2015). The use of ion-selective electrodes for ion analysis, such as Ca and K have many advantages. The sensors are small and allow on-site monitoring, the sensitivity of the ISEs allows for a broad dynamic range; small volumes of solvents are required to perform a sample analysis and continuous sample measurement is possible (Radu et al., 2013). Stability and repeatability of the sensor response are the main concerns when using ISEs as electrode drifts can occur during measurement which can limit accuracy (Kim et al., 2007). Continuous calibration minimizes drift of the ISEs over time and reduces measurement errors due to biofilm accumulation on the ion-selective membrane.

The objective of this study was to design and build a bioreactor for the preparation of chicken manure and the monitoring of the nutrient solution used in hydroponic systems within the PoultryPonics facility. The manure extract was monitored with different ion-selective electrodes and adjusted using inorganic chemicals to meet crop nutrient requirements.

8.2 Materials and methods

8.2.1 Bioreactor setup

Two 200 L metal barrels (55 gal.) previously used to store chicken feed were cleaned and redesigned for the bioreactor (Figure 8.1). In the first metal barrel, the digestion tank, raw manure was added in a meshed bag and was placed in a meshed basket. 2.51 cm (2 in.) PVC tubes were fixed together, and air was pumped from the bottom (EcoPlus 728457 1300 GPH (4920 LPH, 80W) commercial air pump with 8 valves, Hawthorne Gardening Company, Vancouver, WA, USA) that created an air lift and generated water movement. The water from the pump felt back on the mesh bag, aerating and mixing the manure extract solution. Once the solution in the digestion tank was considered mature, after a period of 24-48 hours, a movable submersible pump was used to pump the water to a retention tank, a 18.9 L (5 gal.) plastic pail.

In the retention tank, the manure extract was filtered using gravity and passed through two sieves of 40 and 80 mesh (Brass frame & 304 stainless steel screen, 3" diameter x 1" deep, McMaster-Carr, Elmhurst, IL, USA) and went to the conditioning tank.

The conditioning tank was continuously aerated (NewLife Intensity Oxygen Concentrator, AirSep Corporation, Buffalo, NY, USA) to promote an aerobic environment. The exterior of the tank was isolated with a 1.25 cm (1 in.) foam. Within the conditioning tank was a bucket heater (1,000 W Water Bucket Heater, API Model C742G, Miller Manufacturing, Glencoe, MN, USA) and temperature was maintained between 60-70^{o}C to kill harmful pathogens (Ingham, 2002). Manure extract was sampled and analyzed by ion-selective electrodes and nutrients were adjusted with inorganic fertilizer prior being added to the hydroponic system within the facility.

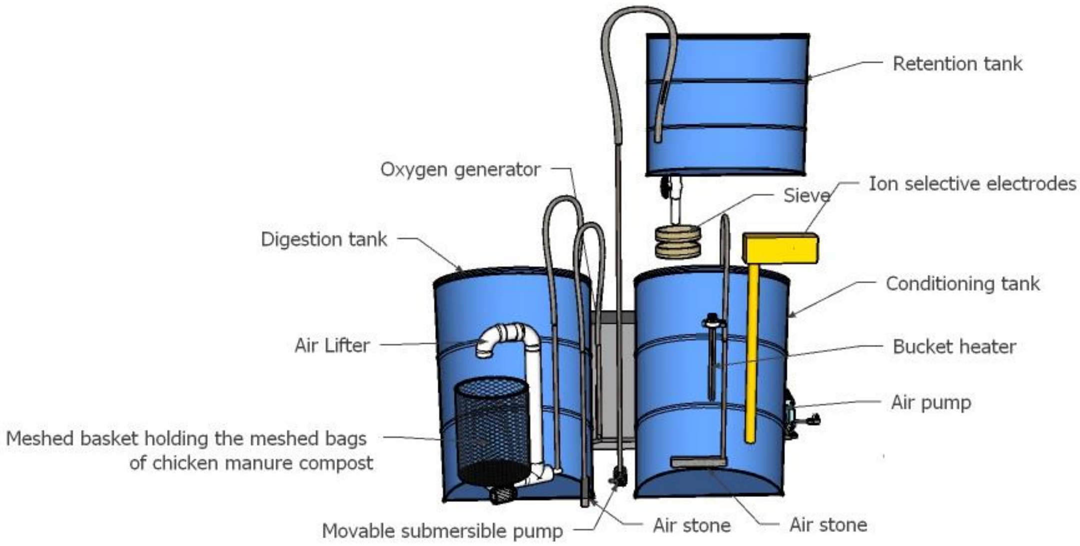

Figure 8.1. PoultryPonics bioreactor design.

8.2.2 Nutrient sampling box setup

A sample of manure extract from the conditioning tank was transferred into the nutrient sampling box (Figure 8.2) with a peristaltic pump (Peristaltic Liquid Pump with Silicone Tubing, Adafruit, NY, USA). The nutrient sampling box, a 3D printed design, had eight openings on top to allow the placement of ion-selective electrodes. There are four 10.2 mm^2 openings on each side for rinsing the solution with fresh water after each measurement (AGPtek® DC 12V 45W High Pressure Micro Diaphragm Water Pump Automatic Switch 3.6L/min, Guangdong, China). A total of four 60.8 mm^2 openings were on opposite sides, two on each side, to allow overflow to exit the sampling box, in case more nutrients were sampled than necessary. There was one 176.7 mm^2 discharge valve (MISOL 1PCS of motorized ball valve G3/4"(BSP) DN20, Zhejiang, China) operated individually at the bottom of the box to empty the nutrient sampling box once the measurements were taken. To hold the nutrient sampling box in one place, two screws were printed, and the design allowed to be held on to a metal bar.

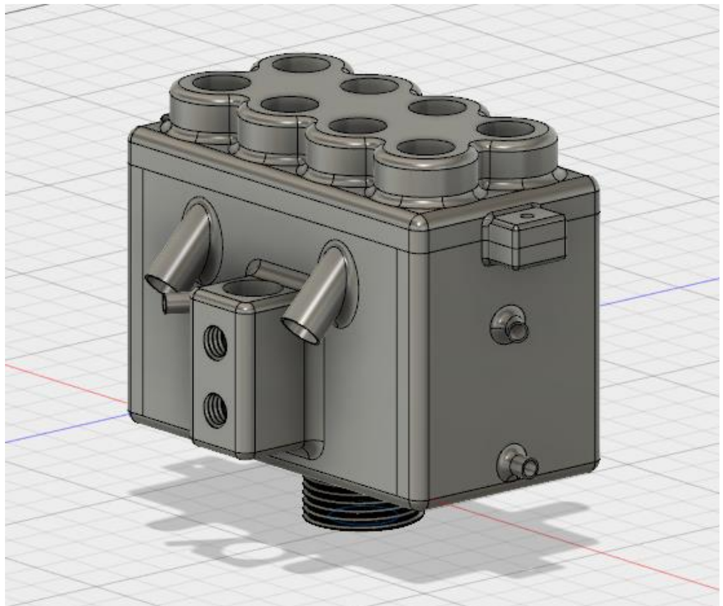

Figure 8.2. Nutrient sampling box used to monitor nutrient solution and to hold the ISEs in place.

8.2.3 Nutrient monitoring box setup

The nutrient measuring box held the ion-selective electrodes (Figure 8.3). For this design, only four ISEs were used: detectION ™ 3031 BN Potassium Combination Electrode, detectION ™ 3041 BN Calcium Combination ISE, detectION ® 3021 Nitrate and detectION 3051 Ammonium Combination ISE (Nicosensors, Huntingdon Valley, PA, USA). All sensors were plastic membrane sensors with a double chamber reference system. All ISEs were connected to a Tentacle Shield (Tentacled Shield, Models T1.15A, T1.16, Atlas Scientific LLC, Long Island City, NY, USA) that was operated by an Arduino UNO atmega328 (Arduino, Somerville, MA, USA). An LCD display screen (20x4 IIC/I2C/TWI LCD Module, SunFounder, Buckinghamshire, UK) displayed the nutrient concentration measured by the separate ISEs. Measurements were taken one ISE at a time to minimize interference. Once the nutrient sampling box and nutrient measuring box were setup, the process flow to determine nutrient concentration was implemented (Figure 8.4).

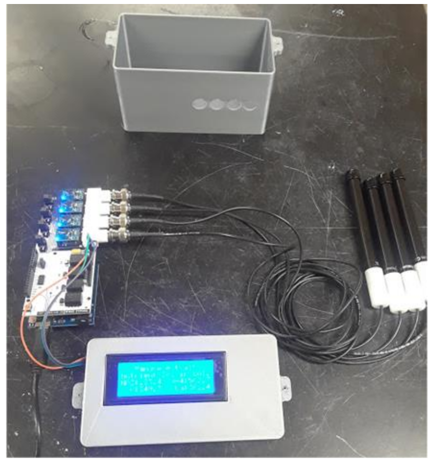

Figure 8.3. Box designed to hold the Arduino Uno with the tentacle shields and the LED screen to display the nutrient content measured by the ISEs.

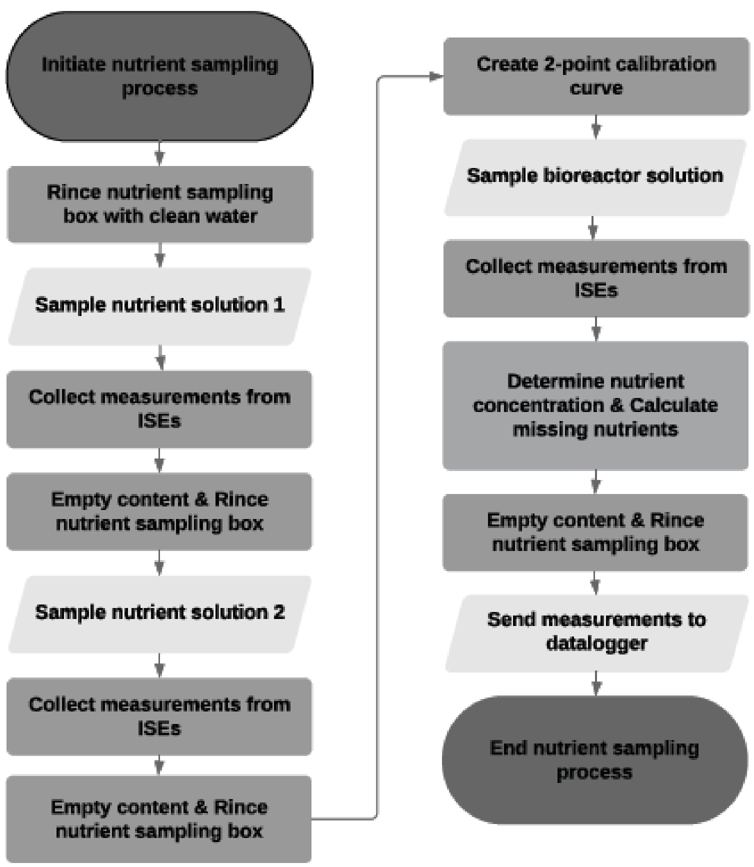

Figure 8.4. Nutrient sampling work flow process

The nutrient sampling workflow process was designed to rinse the nutrient sampling box after each measurement to minimize the buildup of biofilms on the ISEs (Figure 8.4). Two sample nutrient solutions can be used in this setup to create a two-point calibration system prior measuring the sample in the bioreactor solution. The same solution should be used, one at half strength, diluted, and one at full strength, not diluted. The sample nutrient solutions can either be a previously used manure extract with known nutrient concentration or an inorganic fertilizer. The nutrient solutions used in the sample solutions should be close to the values expected in the manure extracts to minimize reading errors.

8.2.4 Bioreactor setup

Initial bioreactor and nutrient sampling box with nutrient monitoring box were setup in the PoultryPonics facility (Figure 8.5 and Figure 8.6). The bioreactor operates in batch, with two main steps; the digestion and the conditioning. Digestion can last from 12 to 48 hours. The digestion consists in infusing chicken manure compost in a highly oxygenated water, allowing the increase of the bacterial activity and nutrient breakdown. The conditioning consists in a filtration removing small particulate matter from the nutrient solution previously brewed, followed by an increase in temperature killing potential pathogens.

Figure 8.5. Picture of bioreactor in the PoultryPonics facility.

Figure 8.5 shows some of the main component of the bioreactor, in blue the digestion tank, in white the insulated conditioning tank in grey the oxygen generator.

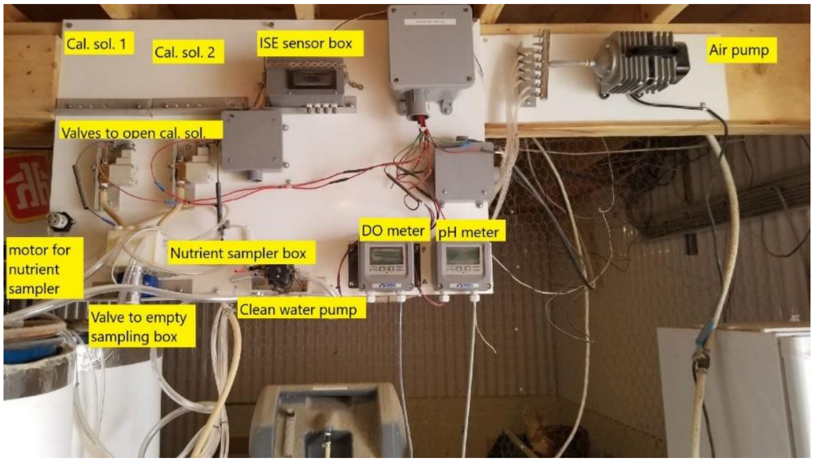

Figure 8.6. Nutrient monitoring device (with labels) used to monitor the nutrient solution.

Figure 8.6 shows the mounting panel for the ion-selective electrodes; this panel includes all the equipment to proceed the cleaning and calibration of the ion-selective electrodes, the sampling of nutrient solution, the nutrient monitoring box to activate all the pumps and the LCD display to display the measurements.

The measurements taken by the nutrient monitoring box were transmitted to a Campbell Scientific CR1000 datalogger (Campbell Scientific, Edmonton, Canada). Additional temperature sensors, dissolved oxygen meter and pH meter were added separately to the datalogger.

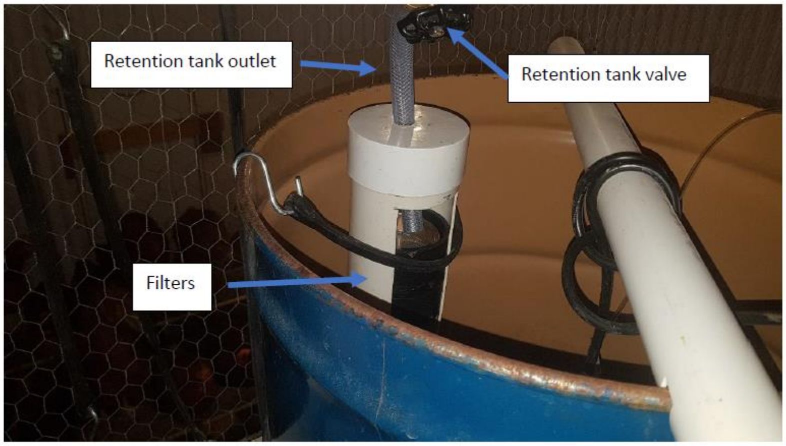

Figure 8.7. Retention tank setup.

8.3 Results and discussion

An initial run was completed using 50 g of fresh manure per L of water. A total of 110 L of solutions were prepared. The manure was left in the digestion tank for a period of 24 h prior being transferred to the retention tank (Figure 8.7). Temperature in the Conditioning tank was kept between 60-70ºC to kill harmful pathogens and was constantly aerated. Due to lack of time, monitoring of the manure extract could not take place, however the system was linked and data collected was stored in the Campbell Scientific datalogger.

8.3.1 Manure nutrient content

Dry chicken manure and water were sampled in the facility. Dry manure was manure found on the floor of the facility and was about one week old. Nutrient content was measured using flame spectrometry (Table 8.1).

Table 8.1. Chicken manure sample collected from the PoultryPonics facility.

Treatment	P (mg/L)	NH_4 (mg/L)	K (mg/L)	Ca (mg/L)
Dry manure	20.5 ± 0.6	104.8 ± 0.5	226.2 ± 3.7	14.6 ± 0.3
Water	0.1 ± 0.0	0.2 ± 0.1	2.8 ± 0.2	25.7 ± 3.0

Once the bioreactor is operational tomatoes or lettuces would be grown in the hydroponic system. A study completed by Premuzic et al. (1998) studied the effect of various nutrient uptake when tomatoes were grown in an organic solution prepared from soil and vermicompost. In their study, the nutrient levels in the soil were 360 ppm for Ca, 540 ppm for K and 5 ppm for P (Premuzic et al., 1998). Chicken manure had higher P, 20.5 ppm, but lower K and Ca levels, 226 ppm and 14.6 ppm respectively. The same study showed that organic solutions release nutrients slowly over time, which optimize nutrient availability during tomato fruit growth (Premuzic et al., 1998). Addition of organic residues or inorganic fertilizers might be necessary to balance the solution to meet plants nutrient requirements.

Ammonium levels in the solution were still high, over 100 ppm, in the dry chicken manure. Nitrate is the preferred form of nitrogen uptake by plants (Shinohara et al., 2011). Contrary to nitrate, ammonium is known to be toxic for plants at higher concentrations (Britto et al., 2001; Savvas et al., 2006; Sonneveld & Voogt, 2009). However, the presence of ammonium at low concentration can be favorable for plant growth. Supply of 4.7% $N\text{-}NH_4$ can stimulate lettuce growth and enhance phosphorus uptake (Savvas et al., 2006). Adams (1997) recommended a $N\text{-}NH_4$ concentration between 10-20%, however, in most common nutrient used in greenhouses, 40% of total nitrogen is in ammonia-plus-urea form nitrogen and is considered ideal for plant growth (Adams, 1997; Nelson, 1991). Use of the bioreactor would promote nitrification and reduce ammonium levels that would no longer be toxic for plants.

Table 8.2. Cost analysis for the setup of the nutrient monitoring device at the PoultryPonics facility in Hay River, Northwest Territories, Canada

Material	Note	Value ($CAD)
Ion-selective electrodes	N-NO_3, N-NH_4, Ca and K	$1,200
3-D printed boxes	For ISEs and for nutrient sampling	$50
Electronic components	Tentacle shield, Arduino Uno, LED display	$300
Pumps & valves	2 pumps for sample intake and water cleaning; valve for emptying box	$300

Overall, the cost of the nutrient monitoring device was around CAD $1,850 (Table 8.2). Most of the cost was associated with the ion-selective electrodes. Analysis in a laboratory would cost for N-NO_3 and N-NH_4 would be CAD $5.00 each and for Ca and K it would be $1.00 each, for a total of CAD $12.00 per sample and does not include shipping fees. For an operational bioreactor, sampling at least once per day, the setup would be paid off in 155 days if the samples were sent to a laboratory analysis. ISEs will give real-time readings over laboratory analysis and with proper maintenance can last over a year before being replaced.

8.3.2 Recommendations

Many tasks need to be completed to optimize the setup, starting with the automatization of the calibration procedure and the establishment of composting strategy for the chicken manure prior being added to the bioreactor to increase its nutrient content. In the nutrient monitoring box, a tare button was added to reduce noise from the readings and needs to be tested.

8.4 Conclusion

Being able to produce local food is one method to reduce food cost in remote locations and solve food insecurity. Transformation of the chicken manure to a nutrient solution will reduce the cost of inorganic fertilizers used in the PoultryPonics facility with the future objective to maintain similar yield levels as inorganic fertilizers. Automation of the bioreactor and the nutrient monitoring will facilitate the deployment of such facilities in remote areas where growers might not have all the technical knowledge.

8.5 Acknowledgement

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Connecting Text

Chapters 1 to 7 provided information on how to prepare and monitor manure extracts used in hydroponic systems. The purpose of these studies was to investigate the questions on which nutrients to monitor in manure extract solutions made from different manure, how to minimize ammonium levels in manure, and, how to balance the manure solution. In Chapter 8, a bioreactor and a nutrient monitoring design was developed and implemented at the PoultryPonics facility in Hay River, Northwest Territories of Canada. This study applied all knowledge gained during these experiences in a real-world model for plant production using chicken manure to prepare the manure extract solutions used in hydroponic systems.

The final chapter provides a summary of all the results found in earlier chapters. Chapter 9 presents the contribution to the scientific knowledge and the application of the outputs towards the use of manure extract as organic fertilizers, the monitoring of the solutions with ion-selective electrodes and the implementation in greenhouses or other controlled environment facilities. At the end of this chapter, several recommendations are proposed which may be helpful for future research in the field of hydroponic systems.

9 GENERAL CONCLUSIONS AND RECOMMENDATIONS

9.1 General summary

The objective of this research was to develop and optimize a hydroponic crop production nutrient supply system that uses animal manure as a source of fertilizer and to implement macronutrient monitoring using ion-selective electrodes. A survey of current fertilizer uses in agriculture showed that organic fertilizers are already being employed. However, the information available on the preparation methods is insufficient and comparison between different studies can be a challenge as experiments are conducted on different plants using different organic fertilizer sources. To date, limited research has investigated the use of organic fertilizers in greenhouses or with hydroponic systems. In the existing studies, the nutrients present in the organic solutions were not consistently measured, and it was difficult to determine if a nutrient imbalance had an impact on plant biomass. In this thesis, manure extract solutions were prepared for hydroponic systems where lettuce and kale were grown in such systems. Nutrients were supplemented to the organic solution, and nutrients were measured remotely and in real-time with ion-selective electrodes to provide information to the grower and thereby optimize the plants growing environment.

Manure extracts were prepared from chicken, cow, and turkey manure at three concentrations: 10, 25 and 50 g/L. Lettuce and kale were grown in ebb-and-flow hydroponic systems with manure extracts, and plant yields were compared to the control Hoagland solution. Highest aboveground dry mass was achieved in the 50 g/L turkey solution for lettuce. Ammonium toxicity likely killed all plants grown in the chicken extract (50 g/L). Nutrient analyses of all manure extract solutions showed a 29% to 79% higher concentration of $N\text{-}NH_4$ and higher total nitrogen concentration than the control. A PCA analysis identified six key nutrients to monitor in manure extracts: ammonium, nitrate, potassium, manganese, magnesium, calcium, and sodium. This study served as an initial investigation on the potential of animal manure as an alternative to inorganic fertilizers in hydroponic systems.

Manure extracts are high in ammonium content, low in nitrate, and are deficient in potassium and sodium. Solid and powder slag cement was added to 50 g/L chicken manure extract solution to balance the nutrient composition. Lettuce and kale were then grown in a hydroponic system with solid slag cement treatment. Solid slag cements increased calcium (38.8 to 57.4 mg/L), potassium (93.4 to 121.2 mg/L) and sodium (26.3 to 54.5 mg/L) content in the solutions. Healthy lettuce and kale plants were grown, but their total aboveground wet mass was 8% and 19% respectively of the control, Hoagland solution. Even if the plants were smaller, none of the plants died in the manure extract solution with the addition of solid slag cement.

Following the study on the addition of solid slag cement to chicken manure extract, the chicken manure extract (50 g/L) was aerated, and molasses was added to the hydroponic solution. Aeration and molasses allowed to reduced ammonium content by 62% within two weeks. Aeration promoted nitrification and resulted in the doubling of the nitrate concentration. However, nitrate levels were still below 10 mg/L after two weeks. Ammonium and nitrate ion-selective electrodes were used to monitor nutrient levels over time. At harvest, the control Hoagland solution produced over six times the aboveground wet mass of kale and over five times for lettuce compared to the chicken manure extract. Given the results of this study, it was determined that additional nutrient requirements, either organic or inorganic, are essential for optimal plant growth and that a pre-processing step would allow to reduce ammonium and increase nitrate concentration in chicken extracts.

The experiments on manure extracts showed that proper assessment of nutrients is necessary throughout the experiment. Three methods (API water test kit, Lachat flow injection instrument and ion-selective electrodes) were then compared to determine the optimal method to measure ammonium and nitrate in the manure extract solutions. Chicken, cow and turkey manure were used to prepare the manure extracts. Additional pre-treatment steps were used for the API water test kit method to reduce the presence of particulates in the solution. Nitrate concentrations were low, below 20 ppm, and were difficult to measure with ISE and API. However, the ISE was the most reliable to measure ammonium in the solutions, with levels at 34.8, 130.6 and 459.0 ppm in cow, turkey and chicken manure respectively. Second order polynomial equations were used to adjust ISE readings. ISEs provides real-time readings over the API water test kit and Lachat readings. ISEs can be easily transported to sampling sites and does not require sample pre-processing prior measurement.

Optimal irrigation was also investigated for plants grown in hydroponic systems. CAN bus systems were developed for irrigation control. Pre-set moisture levels were used to activate water pumps in hydroponic systems. The highest yield was measured in the timed treatment, where moisture varied from 88%-100%, and lowest in the 25%-85% treatment. Regression equations allowed to determine plant characteristics based on initial or end of irrigation moisture levels. This indicated that initial moisture content used to activate the water pump had a significant effect on lettuce and kale biomass.

Preparation of manure extract and monitoring of nutrient in the hydroponic solutions was incorporated in the PoultryPonics facility, a controlled environment where both chickens are raised and vegetables, such as kale and lettuce, are grown year-round. Chicken manure was the main source of fertilizer for the hydroponic system. A bioreactor was built, a nutrient monitoring device was developed using potassium, nitrate, ammonium, and calcium ion-selective electrodes to monitor nutrients in the bioreactor. Even if ISEs have a high initial cost, they remain more affordable than sending samples to a laboratory, and the cost of ISEs are paid off within 155 days. ISEs also provide real-time reading and inform the grower of any nutrient imbalance in the bioreactor.

9.2 Contributions to knowledge

The work presented in this thesis demonstrates the use of manure extracts in hydroponic systems as an alternative to inorganic fertilizers. The experimental methods to prepare and balance the nutrient solution combined with the monitoring of such solutions with ion-selective electrodes can help greenhouses and growers to make the transition towards organic fertilizers. A variety of experiments were conducted, and the main contributions of this research include:

1. The development of a manure-based solution and identification of key nutrients to monitor in the organic fertilizers to prevent nutrient toxicity and nutrient imbalance. Results helped to identify which ion-selective electrodes to purchase for continuous solution monitoring without monitoring all micro- and macronutrients.

2. The effect of the addition of slag cement to manure extracts to balance the nutrient solution. Initial findings determined the amount of slag cement added to the solution based on the volume and concentration of manure extracts prepared. Results showed that healthy lettuce and kale plants can be grown using the solutions.

3. The effect of aeration and the addition of molasses on ammonium and nitrate concentration in manure extracts. Ammonium is known to be toxic for plants at high concentration, and nitrate is the preferred form of nitrate for plants. Monitoring of the solutions with ion-selective electrodes would allow the grower to determine when the manure extract is optimal for plant growth.

4. The investigation and comparison between three methods to measure nitrate and ammonium in manure extract established which methods have the most advantage. Ion-selective electrodes allow real-time monitoring over Lachat and API methods.

5. The development of a CAN bus system for greenhouses capable of monitoring temperature and moisture levels in rockwools, and to activate and turn off water pumps. This methodology would allow to grow plants in greenhouses with specific irrigation needs with minimal change in the programming system. Such a system would be able to detect plant needs faster than currently used general systems.

6. The development of a bioreactor with a nutrient measuring device using ion-selective electrodes that can be integrated into greenhouses or facilities raising animals and growing plants. The bioreactor allows for the transformation of manure to a nutrient solution while the nutrients are being tracked with ion-selective electrodes. Such a system would allow remote food production with minimal use of inorganic fertilizers.

9.3 Further suggested studies

In this research project, organic fertilizer solutions were developed for hydroponic systems. Kale and lettuce were grown to measure the effect on plant biomass. Three different manure sources were used: cow, chicken, and turkey, at three concentrations. Addition of slag cement and molasses, and aeration are necessary to balance the nutrient solution, decrease ammonium content and increase nitrate content. Monitoring in real time of the organic solutions was made possible with ion-selective electrodes with proper calibration. The outcome of this research showed that healthy plants could be grown, Nonetheless, future studies could improve the nutrient solution, the use of ion-selective electrodes and our understanding of the nutrient change over time.

- Kale and lettuce, both leafy vegetables, were the only plants grown during the experiments. Future studies should explore the nutrient effect on fruit-bearing crops. Salt-tolerant crops should be experimented on to minimize the risk of sodium toxicity.
- For each of the manure extract solutions, only three concentrations per manure solutions were prepared (10, 25 and 50 g/L). It is likely that for some manure, such as cow, a solution with a higher concentration than 50 g/L could be tested. Future work should also investigate other sources of farm manure.
- Manure extracts are imbalanced. It is rich in ammonium, low in nitrate, but can be lacking other micro- and macro-nutrients. The solutions could be balanced during the preparation steps by adding either organic or inorganic fertilizers. The results of the aeration of manure extracts, the addition of molasses and slag manure in this research can serve as a basis for future work on the nutrient solution.
- Implementing ion-selective electrodes in greenhouses that will plan on employing organic fertilizers would generate valuable information to the grower and would facilitate the commercialization of organic fertilizers. A comprehensive set of experiments should quantify the yield and quality of certain important greenhouse crops with organic fertilizers when the solutions are monitored with ion-selective electrodes.
- Currently, the grower pre-sets irrigation control in the greenhouse. Use of CAN-bus systems to monitor different crop types would allow greenhouses to grow a variety of crops with minimal change in the primary programming system. Automation of irrigation based on moisture requirement could also help to optimize plant growth.
- Animal manure can be the host of multiple pathogens. Designing a proper sterilization process to minimize pathogen transfer from the manure extract solution to the crop would be necessary. By doing so, plants grown in such systems will be one step closer to being commercialized.

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