



Aerated chicken, cow, and turkey manure extracts differentially affect lettuce and kale yield in hydroponics

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Abstract

Purpose 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* var. 'Red Russian') in hydroponic systems.

Methods 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.

Results The aboveground dry mass of lettuce grown in a 50 g/L turkey manure extract solution was 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%–79% higher concentration of $\text{NH}_4\text{-N}$ and higher total nitrogen 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.

Conclusions 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

Introduction

Hydroponic plant production is a soilless agricultural practice that comprises a nutrient solution and an artificial rooting substrate to support plant growth (Kumar and Cho 2014). Hydroponic systems are mostly employed in greenhouses, where the environment can be controlled and plant production can occur year round (Lee and 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 (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 allow for the cultivation of plants with higher and more uniform nutritional values (Surendran et al. 2016; Suvo et al. 2017).

As organic 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 (Williams and Nelson 2014). In recent years, alternate hydroponic substrates derived from solid municipal waste have been considered (Haghighi et al. 2016). In organic farming, “compost teas”

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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. 2009; Ingham and 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 cinerea*) (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–36-h period that may be further extended (Scheuerell and Mahaffee 2002). Non-aerated compost teas are produced by steeping compost in water for several days to weeks (Ingham and Millner 2006). For both aerated and non-aerated compost teas, fermentation parameters can influence the composition and population of microbial species (Scheuerell and Mahaffee 2002).

In Canada, lettuce and kale were amongst the top five vegetable imports in 2016, representing CAD \$447 million and CAD \$374 million in annual sales, respectively (Agriculture and Agri-Food Canada 2017). During the same period, lettuce grown in Canada generated CAD \$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. 2009; Jack and Nelson 2010; Radin and 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 the 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, the 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). 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.

Materials and methods

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 dH₂O (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 2 weeks with a constant temperature of 25 °C, 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 (Fig. 1).

All plants were harvested and dried 28 days after being transferred to the greenhouse (42 days total). At harvest, the following characteristics were measured: aboveground





Fig. 1 Placement of 24 rockwool cubes with kale and lettuce seedlings after transfer to an ebb and flow hydroponic system

wet mass and the number of mature leaves (≥ 2 cm in diameter). Plant samples were oven-dried at 40 °C for 5 days 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 analyses were performed.

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. 1999). 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.

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 and 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.

Electrical conductivity and pH of manure-derived hydroponic nutrient solutions

EC and pH of each manure extract solution were measured every 2 days 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).

Manure-derived hydroponic nutrient solution analyses

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, Milwaukee, WI, US). Briefly, equal amounts of buffer solution and sample were 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 colour at 660 nm with a flow injection autoanalyzer, according to the manufacturer's instructions (Lachat Instruments 2008a).

The Quickchem method (Lachat Instruments 2008a) was also used to determine the nitrate concentration of each solution. A total of 5 mL 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 (Lachat Instruments 2008a).

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 2008b). 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 and Beare 1993) and according to the manufacturer's instructions (Lachat Instruments 2008b).

Elemental analyses

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 ddH₂O 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 ddH₂O in a 50 mL Falcon tube. The samples were analyzed with the Varian 820-MS ICP Mass Spectrometer (Analytik Jena, Jena, Germany).

Statistical analysis

Principal component analysis (PCA) with JMP version 13.0.0 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 13.0.0 software was used to construct all graphs.

Results and discussion

Plant yield

Lettuce grown in turkey manure extract (50 g/L) solution yielded the greatest aboveground wet mass average (19.1 ± 2.7 g), followed by lettuce grown in the control (Hoagland) solution (17.5 ± 0.7 g) (Fig. 2). Kale grown in Hoagland solution yielded the greatest aboveground wet mass average (11.9 ± 0.5 g), followed by kale grown in the turkey manure extract (50 g/L) solution (6.1 ± 1.0 g). For lettuce, the highest aboveground dry mass was recorded when grown in turkey manure extract (50 g/L) solution (1.4 ± 0.2 g), followed by lettuce grown in Hoagland solution (0.9 ± 0.0 g; $\alpha < 0.05$). For kale, the highest aboveground dry mass was measured in the Hoagland solution (0.8 ± 0.0 g) followed by kale grown with the turkey manure extract (50 g/L) solution (0.6 ± 0.1 g; Fig. 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 – 3.4 ± 0.4 g) and

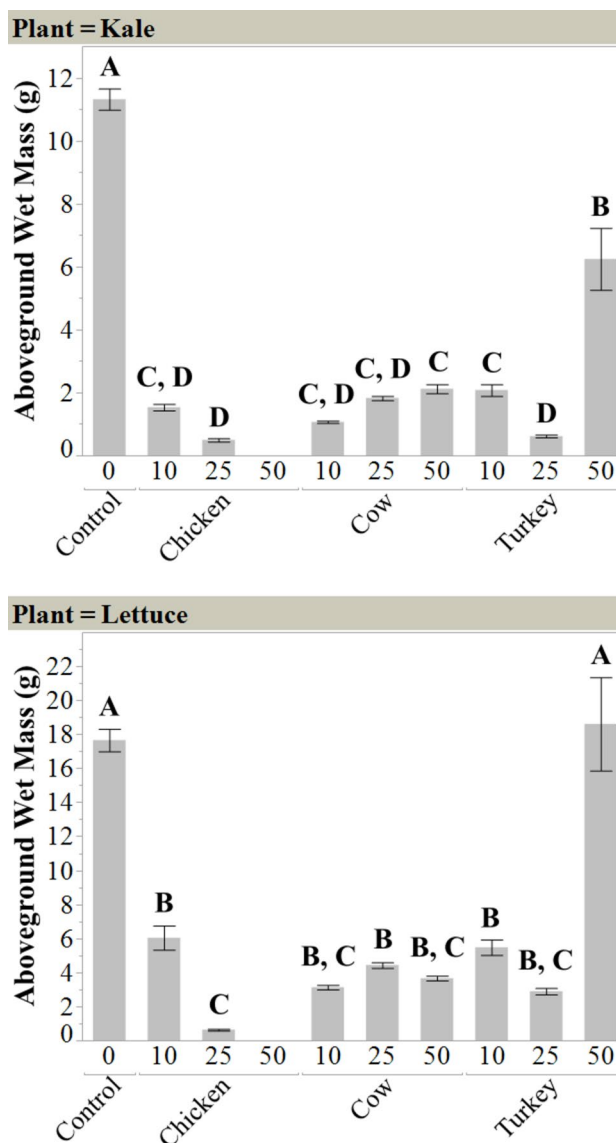


Fig. 2 Mean aboveground wet mass values for manure extract nutrient solutions (\pm standard error), using Hoagland solution as the control. Letters above the columns show HSD statistical significance ($\alpha < 0.05$). Mean values for plants grown in the 50 g/L chicken manure extract solution were not included in this analysis, as none were viable at this concentration

lettuce (3.1 ± 0.1 – 6.6 ± 1.0 g), 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

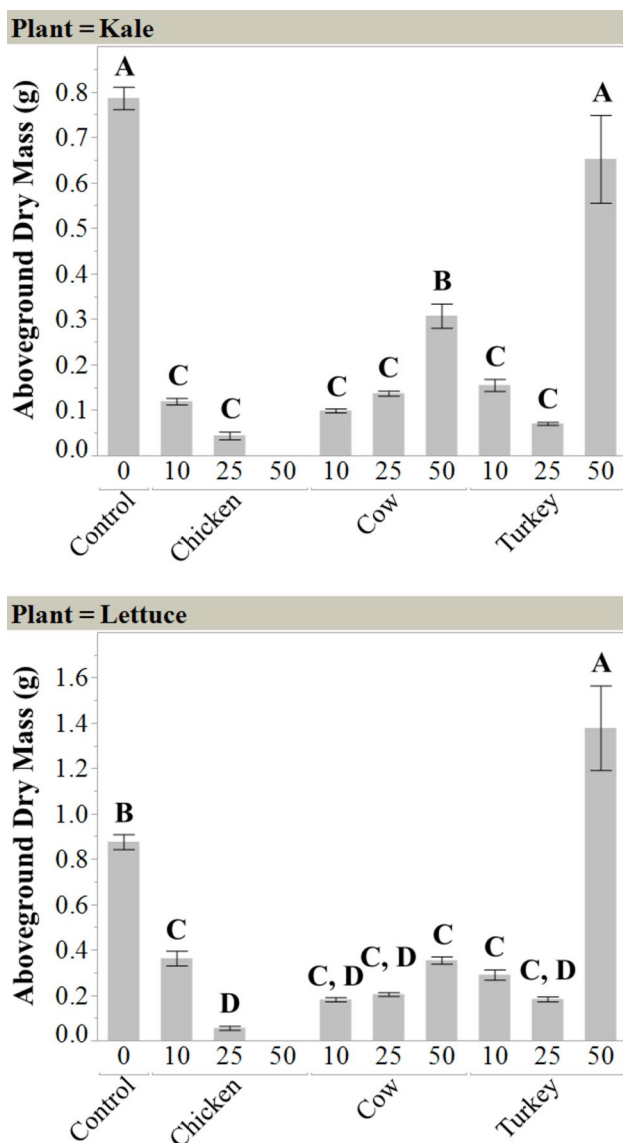


Fig. 3 Mean aboveground dry mass for kale and lettuce grown in different manure extract solutions (\pm standard error), using Hoagland solution as the control. Letters above the columns indicate HSD statistical significance ($\alpha < 0.05$). Mean values for plants grown in the 50 g/L chicken manure extract solution were not included in this analysis, as none were viable at this concentration

greater NO_3 , Mg, and Mn content than plants grown in either chicken or cow manure extract solutions ($\alpha < 0.05$) (Table 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 (Fig. 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 (Food and Agricultural Organization of the United Nations [FAO] 2013). 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 2 and Fig. 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.

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 Fig. 6a, 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 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 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



Table 1 Nutrient variation in the manure extract solutions

Treatment	Concentration	NO ₃		NH ₄		Na		Ca		Mg		Mn	
		mg/L	S.E.	mg/L	S.E.	mg/L	S.E.	mg/L	S.E.	mg/L	S.E.	μg/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 (\pm standard error [S.E.]) for nitrate (NO₃), ammonium (NH₄), sodium (Na), calcium (Ca), magnesium (Mg), and manganese (Mn). These nutrients were selected based on PCA results (Fig. 6). Full-strength Hoagland solution acted as the control. Different letters next to mean values indicate statistical significance ($\alpha < 0.05$)

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.

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 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 NO₃ 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 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 and 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 and 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 and 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. In addition, 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.

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% NH₄-N can stimulate lettuce growth and enhance phosphorus uptake (Savvas et al. 2006), while Adams (1999) recommends an NH₄-N concentration between 10 and 20%. In most commonly used nutrient solutions for greenhouses, however, 40% of total nitrogen is in

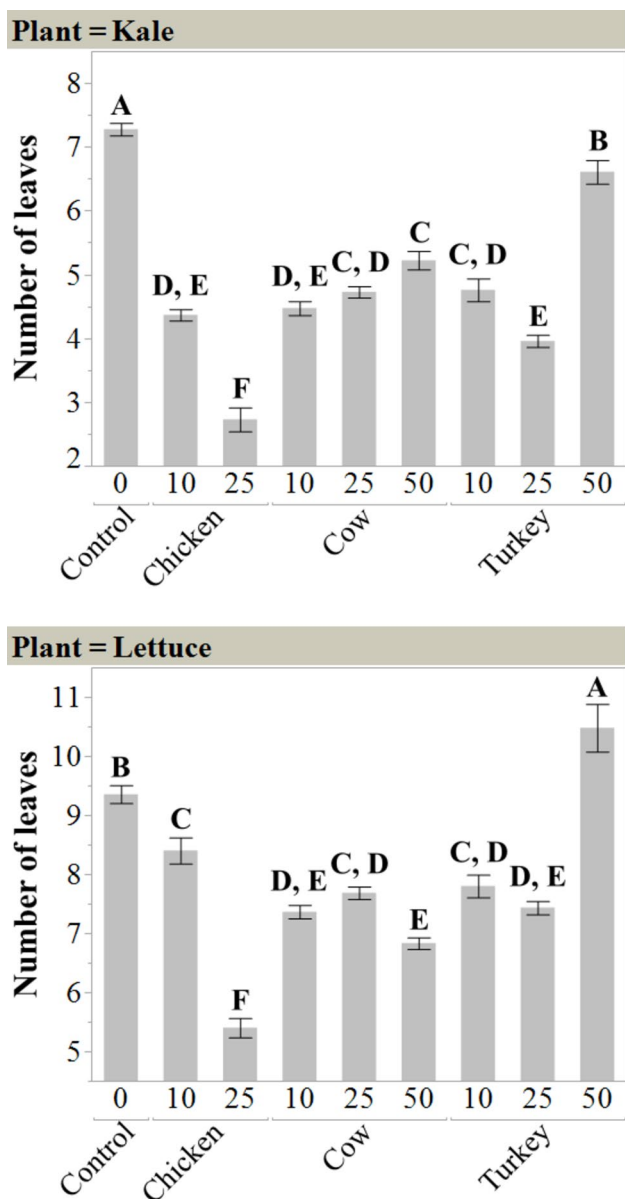


Fig. 4 Mean total number of leaves for kale and lettuce grown in different manure extract solutions (\pm standard error), using Hoagland solution as the control. Letters above the columns indicate HSD statistical significance ($\alpha < 0.05$)

an ammonia-plus-urea form, and this is considered ideal for plant growth (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

Table 2 Target nutrient concentrations in solutions for lettuce (Modified from FAO 2013)

Desired characteristics	Target concentration in the root environment (mg/L)
EC	2.60 (dS/m)
pH	5.6–6.5
Ca	292.6
Mg	38.9
NH ₄	< 10.8
NO ₃	1116.0
Mn	54.9
Na	< 50; For sodium, this is the concentration in water used to prepare the nutrient solution

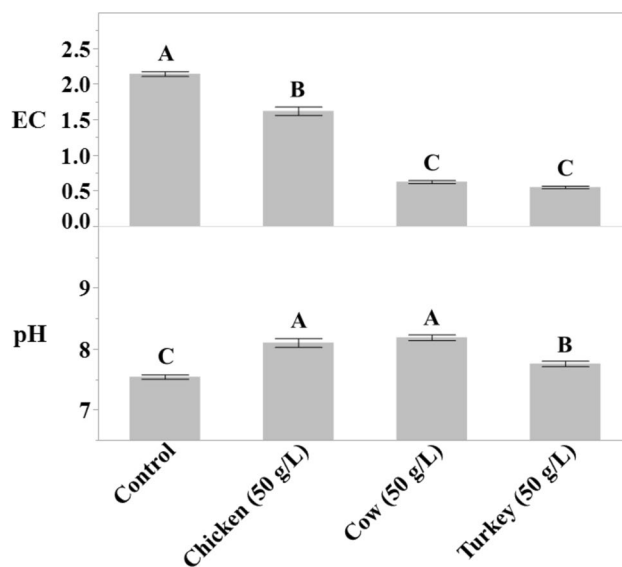


Fig. 5 Mean EC and pH values for each manure extract solution (\pm standard error), using Hoagland solution as the control. Letters above the columns indicate HSD statistical significance ($\alpha < 0.05$)

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 1–6 months contained a lower concentration of ammonium (between 37.1 and 164.2 mg/L) when compared to fresh chicken manure extract solutions (between 100.1 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 and 52.0 mg/L; for chicken, it ranged between 0.5 and 2.7 mg/L).

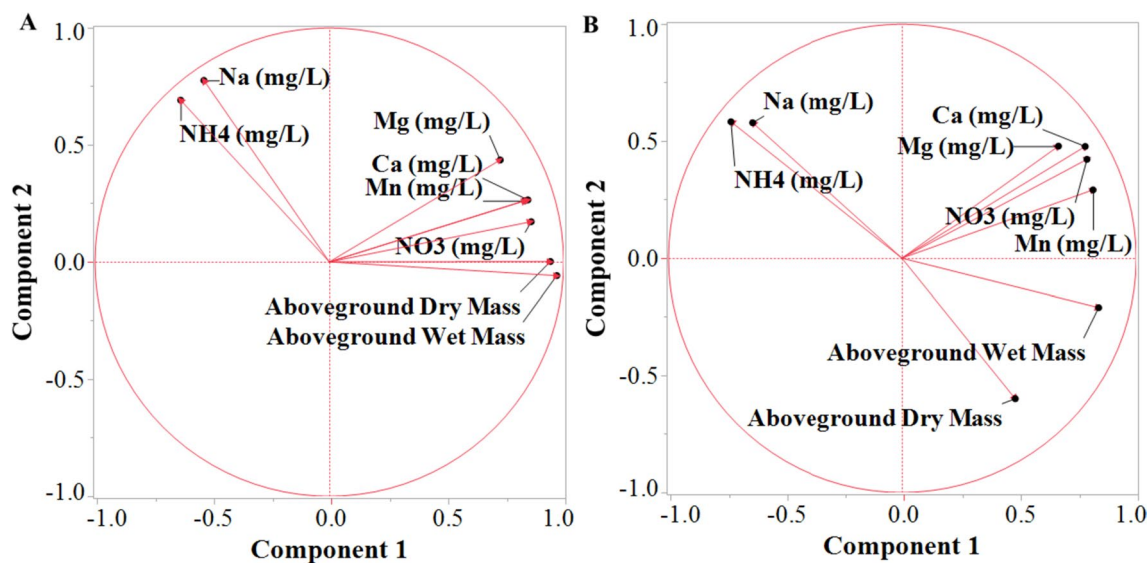


Fig. 6 Principal component analysis of six main nutrients identified for **a** kale and **b** lettuce yield. For both plants, 24 nutrients were originally measured in each manure extract solution. For kale, the first principle component was 65.1% and it correlated with an increase in aboveground wet 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

Tiquia and Tam (1998) used forced aeration on pig litter sludge for 80 days, noting a sharp decrease in $\text{NH}_4\text{-N}$ and an increase in $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$ within the first 20 days of composting (Tiquia and Tam 1998). Cáceres et al. (2006) found comparable results for both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ 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 1999). For lettuce and tomato plants, ideal potassium:nitrogen ratios are 1.7:1 and 2.5:1, respectively (Adams 1999). 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 and 200 mg/L and a phosphorus concentration between 10 and 30 mg/L is acceptable (Adams 1999). 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 1999). For the manure extract solutions in this study, potassium concentrations ranged between 27.2 and 228.2 mg/L, while magnesium concentrations were between 3.5 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 and Nowak 1990; Urban and 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 sweet pepper and cucumber coloration (Janse 1988, 1989). However, a high salt concentration can also limit yellow colouring and leave russetting for tomatoes while maintaining lettuce leaf firmness (Urban and 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 (Fig. 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 (supplementary material; Table 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/g to 1.3 mg/g as the manure extract solution increased from 12.5 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 $[\text{Na}^+]$, $[\text{NH}_4^+]$, $[\text{K}^+]$, and $[\text{Mg}^{++}]$, can induce calcium deficiency in plants (Adams 1999; Meharg and 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 14–15 mg/g dry tissue versus 11 mg/g; supplementary material; Table 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 (FAO 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 1999). 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. 2007). 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 and 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 (supplemental material; Table 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, as suggested levels are estimated at 2.3 mg/day and 1.8 mg/day for men and women, respectively (Przybysz et al. 2017). 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 (Vlamiš and 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 m²) manure died before the end of the experiment. The highest plant yield recorded was grown in a extract solution containing 10 g/L (10 kg per 10 m²) manure, and plant yield decreased at a manure concentration of 25 g/L (25 kg per 10 m²). 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–25 kg per 10 m² (Engelbrecht et al. 2012). Moreover, Muñoz et al. (2004) observed an increase in soil-available phosphorous and fruit yield for papayas when 15 kg per 10 m² (15 t per ha) of chicken manure was applied (Muñoz 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 (FAO 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 μ M for copper (11.4 mg/L) and 260 μ M 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 et al. 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.

Conclusion

Kale and lettuce growth was 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.

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