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Bioponic Cultivation Using Chicken Droppings to Produce Lettuce Plants (*Lactuca sativa rz*) Uncontaminated by Trace Metals

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Abstract: Anthropogenic activities have denatured aquatic, terrestrial, and aerial environments throughout the world in general, and in Lubumbashi in particular, where market garden soils have become uncultivable for many plants. Thus, bioponics could be an effective means of producing uncontaminated vegetables in soilless cultivation, not only reducing the amount of fertilizer used and limiting contamination of agricultural produce but also achieving higher yields than in open-ground cultivation. The overall objective of this study was to implement a new bioponic technique for producing liquid fertilizer from chicken manure and utilize it in the organic hydroponic cultivation of lettuce (*Lactuca sativa var. Lucrecia)* installed on floating raft systems. To achieve this, two types of trials were conducted. The first was aimed at determining the quantities of organic matter to be used in the formulation of nutrient solutions. The second trial aimed to determine the optimal nitrogen concentration to be provided for hydroponic plant growth. Mineralization and/or anaerobic digestion of chicken manure were conducted for 7 days in 200 L barrels. For the first trial, nutrient solutions were created from three different concentrations of chicken manure (0.35%, 3.5%, and 7% dry matter—D.M.). These solutions were then used in bioponic rafts where total ammonia nitrogen (TAN) concentrations were fixed at 150 mg/L. For the second trial, D.M. was fixed at 2.5% for each tested modality, but TAN concentrations varied among them (i.e., 60, 90, and 120 mg/L TAN concentration). Modalities with low D.M. concentration (0.35%) and those with low TAN concentration (60 mg/L) resulted in higher yields than bioponic modalities receiving high concentrations of dry matter or TAN, respectively, for trials 1 and 2. Although the reference chemical solutions generate the greatest yields, bioponic systems operating with chicken manure present a good alternative for the cultivation of vegetables in developing countries with heavily contaminated soils. Indeed, bioponics allows for the production of vegetables in large quantities from animal waste, which does not pose health risks for human consumption. Local vegetable species commonly grown in Lubumbashi should be tested under hydroponic conditions.

Keywords: hydroponics; microorganisms; anaerobic; aerobic; raft

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1. Introduction

Soil is a source of nutrients for plants and, as a substrate, enables plant growth. However, it cannot always play this role, given its degradation mainly caused by human activities. This situation is particularly acute in arid regions and areas of intense mining activity [1–4]. To guarantee food security for a growing population, increasing yields with adapted techniques remains a major challenge in developing countries where access to water and suitable soil is not guaranteed and public agricultural policies are sometimes deficient in feeding their populations [5,6]. This is particularly true in areas where anthropogenic activities are intense, like mining areas where soils have become less conducive to the production of quality vegetables and fruits in recent decades [1,7].

For these areas, hydroponics could be an alternative growing technique to ensure that the grown plants are free from heavy metal contamination. Hydroponics is a soil-less cultivation technique in which plant roots are immersed in a nutrient-enriched solution and eventually maintained by a preferably inert substrate [8]. This method, therefore, has the advantage of dissociating vegetable production and polluted soils. Three important qualities of hydroponics are (i) high yields, (ii) a 40 to 70% reduction in water consumption compared to soil-grown vegetable production, and (iii) its feasibility in areas where access to arable land is limited (due to arid conditions, or simply infertile or polluted soils) [9–14].

In most hydroponics systems, plant roots are immersed in nutritive solutions made from chemical fertilizers [8] derived from petrochemicals. Their mining and manufacturing generate high operating costs and major environmental problems through soil, water, plant, and air pollution [15,16]. For some minerals, their purchase prices can rise rapidly, particularly when the cost of the energy required for production is unstable [17–19]. Thus, in the face of increasing demand for fertilizers, agriculture will encounter numerous problems of fertilizer scarcity between 2050–2100, as mineral deposits are expected to be depleted [20–24]. These problems of food shortage could be exacerbated in developing countries, which are highly dependent on imports of synthetic chemical fertilizers [25–27]. This dependence is set to increase over the next few years, given the depletion of the deposits from which these minerals are extracted, on the one hand, and the poverty levels of the populations living in these countries, on the other. It is therefore essential and urgent to think about innovative and sustainable techniques to overcome these global challenges of chemical fertilizer shortages, and bioponics is one such alternative technique. Also known as biological hydroponics, bioponics involves growing plants in an aqueous medium, with the roots immersed in a nutrient solution derived from the partial or total mineralization of animal manure or plant debris [27–29].

In developing countries, these organic fertilizers can be acquired at low cost [30–32]. As part of the circular economy, it would make it possible to recycle urban waste instead of making it a source of soil and air pollution and diseases (typhoid, malaria, etc.) [33–35]. There are several techniques for producing organic fertilizers, such as compost tea [36–40] and vermicomposting [41,42]. The use of raw materials of animal origin to make compost tea has been shown to have certain advantages, such as the suppression of certain plant diseases [42–48].

In recent decades, poultry production has surged in response to the growing demand for meat and eggs from both urban and rural populations. The chicken droppings generated by this industry can serve as a multi-purpose resource for agriculture, offering benefits in terms of fertilization, composting, sustainability, and cost. Additionally, this high demand for chicken meat and eggs has encouraged residents to engage in poultry farming, leading to significant animal waste production. Chicken droppings can enhance crop productivity, soil health, and environmental sustainability through their rational use, while also protecting the environment from various sources of pollution [49–51].

This study is part of a more global project, which aims to find sustainable solutions to the various environmental problems facing urban agriculture in Lubumbashi. Firstly, the contamination of soil was characterized, by water and plants in the market gardens of Lubumbashi [1]. The results showed that the gardens were low, medium, and high in trace metal contamination and that the vegetables were, therefore, highly contaminated. Secondly, organocalcareous soil improvers were applied to clean up the soil. However, vegetables grown from these soils still presented trace metals above the limits imposed by the FAO, demonstrating that the soil improvers did not help in reducing the mobility and bioavailability of heavy metals [52].

This study aimed to analyze the impact of organic fertilizer produced from chicken droppings on yield and trace metals for lettuce grown in bioponic systems. The chicken droppings were chosen based on their availability in the Lubumbashi region, where intensive and family farming is increasingly popular with the city's inhabitants, to offset the need for staple foods particularly poultry imported from neighboring countries. This study should help in understanding whether these fertilizers are promising for soilless crops run by populations living in the city of Lubumbashi in the D.R. Congo, which faces heavy soil pollution.

To do this, two experiments were set up. The first experiment was implemented to understand the impact of varying amounts of fresh chicken droppings on the preparation of bioponic nutrient solutions and, subsequently, its impact on lettuce yield. The second experiment consisted of determining the optimum nitrogen concentration of the bioponic nutrient solution to optimize bioponic vegetable production. Both yield and quantification of heavy metals were studied in this second experiment to better understand whether this solution is a viable alternative to grown vegetables.

2. Materials and Methods

For both trials performed, a bioponic system was used. The sequence of steps taking place during a bioponic production is described in (Figure 1). These steps were identical for both performed trials.

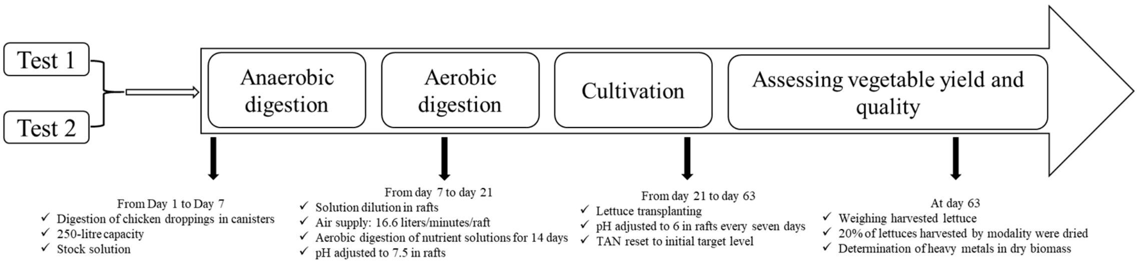

Figure 1. Schematic diagram of bioponic nutrient solution production.

The difference between the trials resides in the preparation of nutrient solutions used. In the first trial, varying amounts of dry matter of chicken droppings were used during the preparation of the nutrient stock solution. In the second test, whilst the dry matter of chicken dropping was kept constant, the total nitrogen of each solution used was varied.

2.1. Plant Material and Growth Conditions

The trials were carried out in a shadehouse located in the Biofortification, Defence, and Crop Valorization (BIODEV) research unit of the Faculty of Agronomic Sciences at the University of Lubumbashi, D.R. Congo.

The lettuce seeds (*Lactuca sativa var. Lucrecia rz*) were obtained from the Laboratory of Integrated and Urban Plant Pathology at Gembloux Agro-BioTech, Université de Liège, Belgium. These lettuce seeds were sown in 36 × 36 × 40 mm rockwool cubes, Grodan, Roermond, Netherlands. Lettuce plants were grown under ambient light conditions and at an average temperature of 20 °C (Figure A2). Eight days after germination, vigorous seedlings with 2–3 true leaves were transplanted onto 2 × 1 m floating rafts, at a rate of 36 plants per floating raft, in a 5 cm diameter hydroponic basket. Lettuces were harvested 42 days after being planted in the rafts (on Day 63 according to Figure 1). All rafts were made of recycled wood, covered with polyethylene bags containing 600 L of nutrient solution, and homogenized by a 950 L/h submersible pump in continuous operation (Sicce, Pozzoleone, Italy) (Figure 2).

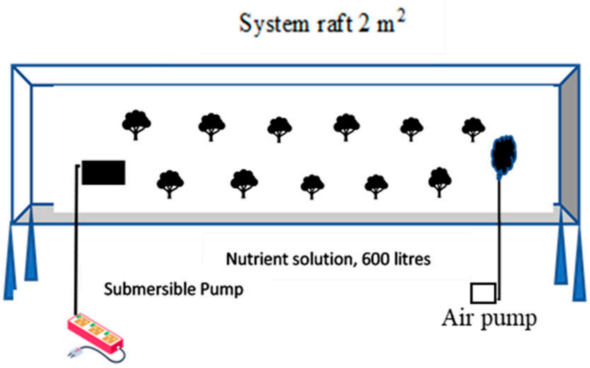

Figure 2. Raft system, organic hydroponic cultivation of lettuce plants.

2.2. Biofilter Preparation

Two weeks before the anaerobic digestion of the chicken manure, bio-balls composed primarily of clay pellets, plastic caps, and biomedia (small plastic cylinders) were prepared in a 100 L capacity tank to ensure the proper development of nitrifying bacteria responsible for the nitrification process of the manure in the rafts. A mixture of 2.5 kg of well-decomposed fresh manure and 2.5 kg of mature compost was combined in 60 L of water, in which 25 L of bio-balls enclosed in a mosquito net were placed. This mixture was maintained under aerobic conditions with an airflow rate of 1.5 L/min into the tank containing the bio-balls for two weeks. The prepared 25 L of bio-balls were then divided into twelve parts, corresponding to the number of rafts (Figure 3).

Figure 3. Preparation of aerobic biofilters (biomedia, plastic plugs, and clay ball).

2.3. Production of Stock Solution from Chicken Droppings

Thus, chicken droppings were chosen as the organic material for these experiments because it was readily available from both small and large poultry farmers in the city, and it was cheaper than mineral fertilizers [53]. The chicken droppings were purchased from an industrial poultry farm located about 15 km from the Faculty of Agricultural and Environmental Sciences at the University of Lubumbashi. For the production of nutrient solutions, all treatments (0.35%; 3.5%, and 7% dry matter D.M.) were repeated three times, i.e., three cans per treatment leading to a total of nine cans for trial 1 (Table A2). For trial 2, six repetitions (six cans) of the single modality (2.5% dry matter—D.M.) were produced (Table A3). To determine the dry weight of chicken droppings, ten 100 g samples of fresh chicken droppings were taken, weighed, and placed on aluminum plates in an oven at 40 °C for 48 h and then at 105 °C for 24 h (Table A1).

Once the stock solutions were prepared, the mineralization and/or anaerobic digestion of chicken droppings was carried out over a period of 7 days in 200 L canisters.

Following this anaerobic digestion, the nutrient solutions were filtered to remove large particles using a 500 µm mesh sieve, followed by a screening cloth. Only after this filtration process were the nutrient solutions supplied to the rafts.

These solutions were diluted to reach the desired TAN (total ammonia nitrogen). In the first trial, each stock solution was attributed to one raft. Although the chicken dropping concentration is variable (dry matter (%) in Table 1), the TAN (total ammonia nitrogen) concentration was fixed to 150 mg/L per raft before anaerobic digestion for all modalities, which was considered to be the highest concentration of nitrogen that plants can absorb in hydroponics [8]. On the contrary, for the second trial, the initial chicken dropping concentration was identical between modalities, but TAN concentrations were fixed to three different values before anaerobic digestion (i.e., 60, 90, and 120 mg/L TAN). This information is summarized in the table below (Table 1).

Table 1. Summary of two bioponic experimental setups with lettuce cultivation (*Lactuca sativa rz*).

Types of Tests	Dry Matter (%)	TAN (mg/L) Target
Test 1	0.35	150
	3.5	150
	7	150
Test 2	2.5	60
		90
		120

After dilution of the bioponic solutions in the twelve rafts, the latter received 2 L of biomedia and 16/6 L/min of air. Each raft then ran empty for 14 days to allow aerobic digestion to take place (from day 7 to day 21 according to Figure 1).

For both trials, lettuce was also grown with a reference nutrient solution of Hoagland to assess the yields and quality of lettuce produced in hydroponic cultures [8]. Given that this reference solution did not require any aerobic digestion, this one was only implemented in the rafts 14 days after the chicken-dropping prepared solutions. For this reference solution, TAN concentration was fixed at 150 mg/L for the first trial and 120 mg/L for the second trial.

2.4. Aerobic Digestion of Nutrient Solutions in Hydroponic Raft Systems before Crop Transplanting: Empty Circulation Phase

The solution diluted in 600 L of water per raft will continue the mineralization process aerobically for 14 days in rafts covered with an impermeable polyethylene bag. Once the bioponic nutrient solution had been diluted in the rafts, the 25 L of biofilter prepared (see Section 2.2) was divided equally in each raft. The bio-balls were left in the rafts until the end of the experiment.

2.5. Lettuce Cultivation and Control of Parameters

Before the cultivation phase, the water volume was restored to 600 L (i.e., the initial water level of the raft) with the chicken manure-based nutrient solutions and the reference chemical solution. Water was added here to counter the loss related to evaporation which took place. Lettuce (*L. sativa Lucrecia rz*) seedlings were then transplanted in each raft. The pH was then controlled and corrected if necessary to reach the desired pH of 6: sulfuric acid (H_2SO_4) was diluted to 10% in case of alkaline pH, and sodium hydroxide (NaOH) 3 N was used when in acid pH situations. Electroconductivity (EC) was also measured using a conductivity meter. Every seven days, the desired TAN concentrations (60, 90, 120, and 150 mg/L) in the rafts were adjusted to reach the initially implemented TAN levels (Table 1) to ensure optimal growth of the lettuce plants until the end of the trials. The TAN adjustment was conducted after analyzing the prepared nutrient solutions with a

HANNA brand spectrophotometer to determine the amount of nitrogen absorbed by the plants and the amount evaporated. Electrical conductivity, pH, and TAN concentration were monitored throughout the cultivation period. Every seven days, measurements were taken in the rafts of each of the tested treatments in trials 1 and 2, respectively, until the end of the trials.

2.6. Sample Characterization

2.6.1. Trace Metal Quantification in Chicken Droppings Raw Material

Essential elements (Mg and Ca) and trace metals were determined at the agro-pedological laboratory of the University of Lubumbashi and the laboratory of the Office Congolais de Contrôle (OCC). The extraction consisted of taking 3 g of dried chicken droppings powder and 28 mL of aqua regia. Paper filters were used to filter the extract, which was then diluted with demineralized water and digested for 20 min at 175 °C in a microwave digestion vessel. Characterization of the chicken droppings solution can be found in Appendix A Table A1. Quantification of trace metals (Cu, Co, Cd, Pb, Zn, Fe) in chicken droppings using Perkin Elmer's Optima 7000 DV ICP-OES spectrometer (PerkinElmer, Inc., Shelton, CT, USA) was performed according to the method developed by [54]. The sanitary quality of the chicken manure used in the production of bioponic nutrient solutions was determined. These chicken manures contained trace metal elements; however, these levels did not exceed the limits authorized by the WHO for its use in open-field agriculture. Of all the trace metal elements analyzed, only zinc slightly exceeds the toxicity threshold of 300 mg/kg of Zn permitted for agricultural soil, unlike other trace metal elements such as Cu, Co, Pb, Cd, and Fe, which are below toxicity thresholds. Ultimately, the chicken manures used pose no risk of contamination to the nutrient solutions on the one hand and the bioponic lettuces on the other.

2.6.2. Physico-Chemical Characterization of Nutrient Solutions

To determine the quality of the nutrient solutions, physicochemical analyses were carried out every week, from the start of digestion to the end of the trials (harvest), using a HANNA HI83300 multiparameter spectrophotometer (HANNA Instrument, Saint Laurent de Mure, France). More specifically, these chemical analyses concerned the control of NPK in its various forms (NH_3-N; NH_3^-; NH_4^+; NO_3-N; NO_3^-; PO_4^{3}; P_2O_5; P; K; K_2O; EC; and pH) and this for all modalities for both trials. During the digestion of chicken manure, samples of highly concentrated nutrient solutions were taken. The collected solution was diluted before performing analyses with a spectrophotometer. During cultivation, the targeted TAN (total ammonia nitrogen) was adjusted weekly by adding concentrated TAN solutions to the rafts.

2.6.3. Heavy Metals Characterization in Harvested Lettuce

The determination of trace metals in the dry matter of lettuces harvested after the trials was carried out using the AOAC (1990) method. From a total of 36 lettuces per raft, a representative sample of each raft (20%) was dried at 105 °C for 72 h. In this way, all replicates of each modality were mixed to form a composite sample for analysis. A one-gram dry matter sample of the composite sample was taken and placed in a 250 mL digestion tube and mixed with 10 mL of concentrated HNO_3. This mixture was then boiled for 30 to 45 min to allow oxidation of all the elements. After cooling, 5 mL of 70% $HClO_4$ and the mixture were boiled until dense white fumes appeared. Next, 20 mL of distilled water was added and the mixture was brought back to a boiling state to remove the fumes. The heavy metals (Cu, Co, Cd, and Pb) present in the vegetables were determined by acid mineralization (HNO_3 + $HClO_4$), and measurements were carried out by flame atomic absorption (FAA) [55,56].

2.6.4. Evaluation of Lettuce Crop Yields

Forty-two days after lettuce transplantation, all plants from each raft were weighed on a precision scale to determine the lettuce crop yields on a per-modality basis (Figure A3).

2.7. Statistical Analysis

Yields in both trials were analyzed using one-way ANOVA (fixed factor was % D.M. in the first trial and TAN content in the second). One-way ANOVA was also performed on heavy metals data in the second trial. When the means were significantly different ($p < 0.05$), a Tukey–Kramer test was performed. One-way ANOVA and a subsequent post-hoc test were elaborated using Minitab 19 (Minitab Inc., State College, PA, USA).

NH_3-N; NH_3^-; NH_4^+; NO_3-N; NO_3^-; PO_4^3; P_2O_5; P; K; K_2O; EC; and pH, on the other hand, were analyzed using a two-way mixed ANOVA (within-subject factor was time and between-subject factor was % D.M. and TAN content in the first and second trial, respectively). If a significant interaction was observed between the two factors, Bonferroni correction for multiple comparisons within each time group was performed. When this was not the case, but a significant main effect was still obtained, Bonferroni correction was also used. Two-way mixed ANOVA and subsequent post-hoc tests were performed in R (R 4.3.2 software, R Development Core Team, Boston, MA, USA).

3. Results

3.1. Assessment of the Impact of Chicken Manure Dry Matter on Bioponics (Trial 1)

3.1.1. Physico-Chemical Parameters of the Nutrient Solutions in the Tanks from Trial 1

During anaerobic digestion TAN, pH, and EC were monitored in all tanks for each of the nutrient solutions modality (0.35% D.M., 3.5% D.M., and 7% D.M.).

In all three cases, significant interactions between these two factors (time and % D.M.) were observed ($p < 0.05$).

In the case of TAN (Figure 4a), it can be said that similar behaviors were observed on days 28, 35, and 42 between rafts that were fed with nutrient solutions of 0.35% D.M. and 3.5% D.M. On the other hand, TAN for the 7% D.M. nutrient solution was significantly greater at all times except day 49.

Electroconductivity (Figure 4b) appears to be the greatest for a nutrient solution at 3.5% D.M. at the beginning of the aerobic phase. Solution 0.35% D.M. gradually increases with time and becomes significantly greater than both 3.5% and 7% D.M. at day 49. Lastly, although overall stable around pH 6, it can be seen from (Figure 4c) that the pH obtained for solution 0.35% D.M. is several times greater than that of the other solutions (e.g., significantly greater than that of 7% D.M. at day 21, 28, and 42). Some biotic and abiotic parameters that could influence the results were not controlled, including temperature, light, and microorganisms in the nutrient solutions and rafts. This decision was made to better align our studies with the actual conditions faced by users.

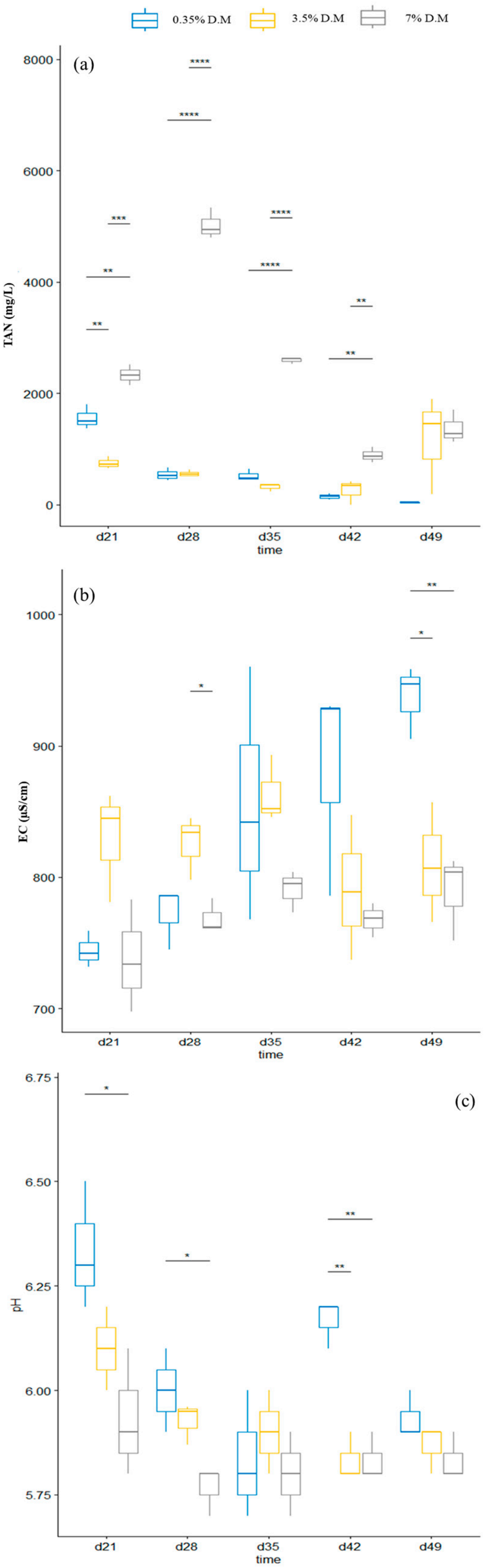

Figure 4. Effect of dry matter on TAN (mg/L) (**a**), electrical conductivity (µS/cm) (**b**), and pH (**c**) of bioponic nutrient solutions manufactured from trial 1. Legend: d: days of observation; '****': Extremely significant difference ($p < 0.0001$); '***': Very significant difference ($p < 0.001$); '**': Highly significant difference ($p < 0.01$), "*": Significant difference ($p < 0.05$); EC: electroconductivity; TAN: total ammonia nitrogen.

3.1.2. Evolution of Nutrient Solutions in the Rafts during the Cultivation of Bioponic Lettuces from Trial 1

The results in (Figure 5) showed that as the culture time increased, concentrations of NO_2-N, PO_4^{3-}, and TAN also increased until reaching their peak on the 35th day, after which concentrations decreased in all bioponic modalities except for the mineral modality (Figure 5a,e). However, treatments composed of 3.5% and 7% dry matter of chicken manure produced the highest amounts of these nutrients compared to the treatment with 0.35% dry matter. For NO_3-N, on the other hand, the highest concentration was obtained on day 21, just after transplanting, and, consequently, nitrate concentrations overall decreased with increasing days of cultivation (Figure 5b). Furthermore, TAN and K contents reached their peak concentrations on the 21st day and decreased as the culture time increased. The best modalities that produced high quantities in TAN were those with 7% dry matter of chicken manure (Figure 5d,f).

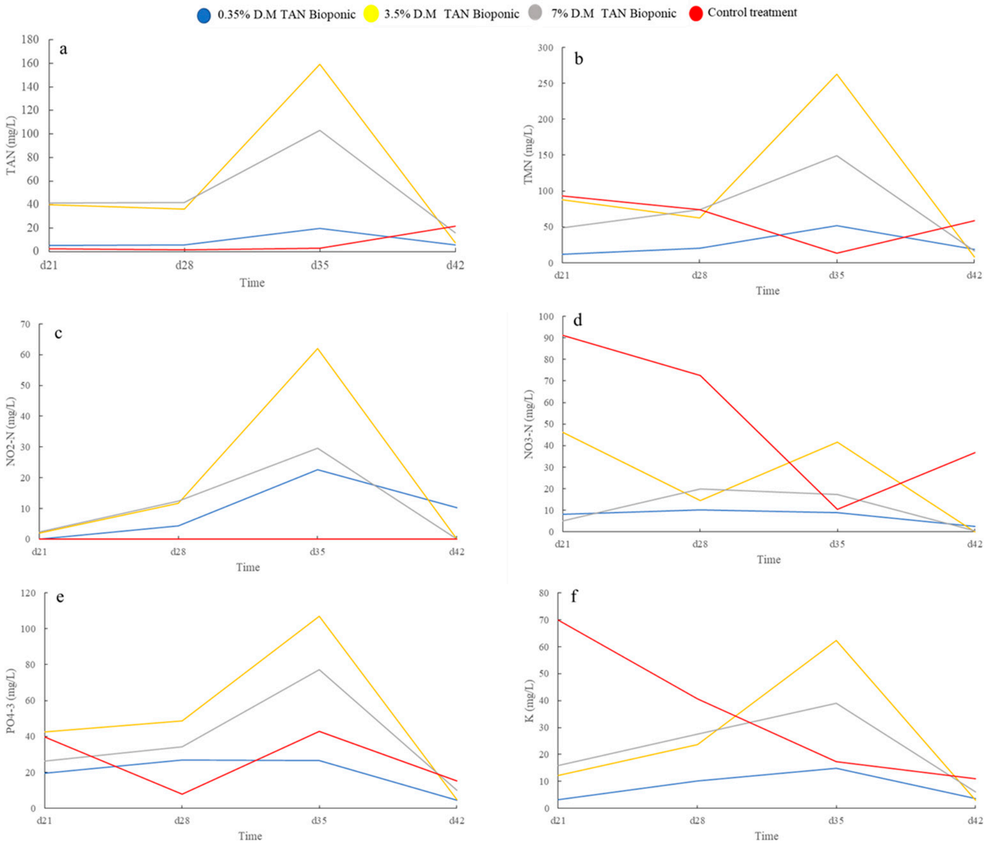

Figure 5. Evolution of NPK levels in the nutrient solutions in the rafts during the cultivation phase of lettuces from trial 1. Legend: d: days of observation, TAN: total ammonia nitrogen (mg/L) (**a**); TMN: total mineral nitrogen (sum of NO_3-N, NO_2-N-, and TAN) (mg/L) (**b**); NO_2-N: nitrite nitrogen (mg/L) (**c**); NO_3-N: nitrate nitrogen (mg/L) (**d**); PO_4^{3-}: phosphate-phosphorus (mg/L) (**e**) and K: potassium (mg/L) (**f**).

3.1.3. Assessment of the Yield and Sanitary Quality of Bioponic Lettuces for Trace Metals (TME) in Trial 1

The analysis of variance shows significant differences between the various modalities applied in hydroponic cultures ($p < 0.05$) in terms of lettuce yield. The significant difference resides between the reference nutrient solution, which demonstrated higher productivity, and the nutrient solutions based on chicken manure (Table 2). However, the analysis of variance shows no significant difference between the modalities with chicken manure. The modality with low dry matter concentration (0.35% D.M.) yielded higher biomass than the other two organic modalities with high concentrations of dry matter. Regarding the sanitary quality of lettuce produced in hydroponics, results show that lettuce grown with a bioponic solution poses no danger to human consumption as the levels of trace elements found in lettuce biomass are below the threshold recommended by the FAO/WHO for human consumption of vegetables. The results of this trial demonstrate that variation in dry matter for the formulation of nutrient solutions does not necessarily influence the increase in crop yields. Thus, in the subsequent trial, the aim will be to test whether, for the same concentration of dry matter for the formulation of nutrient solutions, variation in TAN (total ammonia nitrogen) in the rafts could significantly influence crop yields.

Table 2. Effects of nutrient solutions on yield and health quality of hydroponically grown vegetables in trial 1. Legend, 0.35% dry matter; 3.5% dry matter; 7% dry matter; mineral nutrient solution.

Treatments	**Yield Trial 1 (g)**	**Trace Metals (mg/kg)**					
		As	**Cd**	**Co**	**Cu**	**Pb**	**Zn**
0.35% D.M.	3074.16 ± 57.1 ^{b}	0.05	1.54	0.821	8.51	3.028	18.8
3.5% D.M.	1356.3 ± 581.7 ^{b}	0.8	0.98	0.68	5.73	1.889	22.1
7% D.M.	1702.8 ± 1268.9 ^{b}	0.06	1.05	0.92	6.65	5.868	23.5
Control treatment	8509.9 ± 1405.3 ^{a}	0.9	0.88	0.56	5.89	3.145	15.9

Treatments with at least one common letter do not have a significant difference.

3.2. Assessment of the Impact of Total Ammonia Nitrogen (TAN) Concentration on Bioponics (Trial 2)

As mentioned previously at the end of the first trial, the results showed that the amount of dry matter introduced during the anaerobic manure digestion did not significantly influence the lettuce crop yields. Therefore, in this second trial, it was decided to vary the TAN concentration of the nutrient solution in the rafts to evaluate its impact on lettuce crop yields. For this trial, 2.5% dry matter was chosen for the preparation of nutrient solutions using chicken manure, as previously applied by [57].

During the anaerobic phase, physicochemical parameters such as pH, EC, and TAN were monitored in the nutrient solutions (Appendix A Figure A1).

3.2.1. Evolution of Nutrient Solutions in the Rafts during the Cultivation of Bioponic Lettuces from Trial 2

Following the anaerobic digestion phase, the formulated nutrient solution was redistributed in the rafts, and the TAN concentration was varied according to three modalities: 60, 90, and 120 mg/L of TAN (Table 1). During the lettuce growing phase, chemical parameters such as TAN, TMN, NO_2-N, NO_3-N, NO_2-N, PO_4^{3-}, and K in the rafts were monitored every 7 days to adjust the TAN concentration to the desired level for both bioponic modalities and the chemical reference modality (Figure 6).

Parameters monitored throughout lettuce cultivation are shown in Figure 6. Except for the results obtained for NO_2-N, TAN, and TMN, for all other results, a significant interaction was observed between the time of cultivation and TAN content.

In the case of NO_2-N, TAN, and TMN contents found in the rafts, a significant main effect of time was present. Bonferroni multiple comparison was performed to compare the overall effect of time. It appears that the impact of time is similar in all three cases, with a significant decrease between day 28 and day 35.

On the other hand, observations made regarding the overall impact of the nutrient solution TAN content on the results are variable. For the NO_2-N content and the TAN content, it appeared that all TAN content modalities were significantly different from each other, with the exception of the reference solution and the chicken-dropping nutrient solution fixed at TAN 60 mg/L. No significant effect of TAN content was observed on TMN results.

For the NO_3-N, PO_4^{3-}, and K contents in the rafts during the culture period, there is an interaction between the culture time and the TAN content. In the case of PO_4^{3-}, an overall decrease in phosphate content in the raft fed with the three bioponic solutions can be observed. However, the bioponic solution with a TAN content of 90 mg/L slightly increased between days 21 and 28 before decreasing again. In the case of the mineral solution, the phosphate content remains low and ultimately increases between days 35 and 42. In the case of the potassium content, the Bonferroni multiple comparisons showed a significant difference between modalities at all culture times except on the 35th day where there are no significant differences. It is observed that there is a decrease in K as the culture time increases for all modalities except for the 60 mg/L modality. Regarding the NO_3-N content, it appears that the mineral solution displays the greatest quantities on day 21. In the following days, this one decreases and reaches NO_3-N quantities comparable to the bioponic solutions modalities. As for potassium content, it appears for the nitrate content that the mineral solution and bioponic solution both at 120 mg/L yield the greatest results (Figure 6).

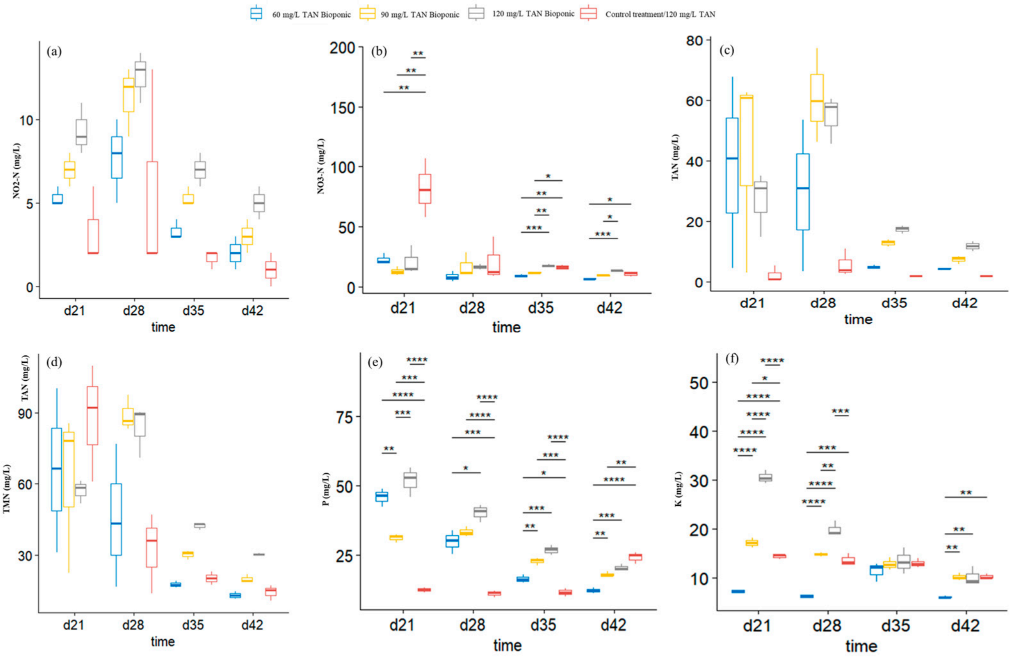

Figure 6. Effect of nutrient solutions and different observation dates on the chemical parameters of nutrient solutions in the rafts of trial 2. Legend: d: days of observation; '****': Extremely significant difference ($p < 0.0001$); '***': Very significant difference ($p < 0.001$); '**': Highly significant difference ($p < 0.01$), "*": Significant difference ($p < 0.05$); EC: electroconductivity; TAN: total ammonia nitrogen; NO_2-N: nitrite nitrogen (mg/L) (**a**); NO_3-N: nitrate nitrogen (mg/L) (**b**); TAN: total ammonia nitrogen (mg/L) (**c**); TMN: total mineral nitrogen (sum of NO_3-N, NO_2-N, and TAN) (mg/L) (**d**); P: phosphate-phosphorus (mg/L) (**e**) and K: potassium (mg/L) (**f**).

3.2.2. Impact of Nutrient Solution TAN Content on Lettuce Yields and Health Considerations

The results of yield and concentrations of trace metal elements are presented in (Table 3). After the analysis of variance, it appears that there is a significant difference between the applied modalities ($p < 0.05$) regarding lettuce yields. The mineral modality was found to be more productive than the bioponic modalities. No significant difference was observed between the modalities of nutrient solutions based on chicken manure; the modality with low TAN concentration (60 mg/L) offered higher yields than the other two bioponic modalities (90 and 120 mg/L). Regarding the accumulation of metals in lettuce biomass, the analysis of variance shows no significant difference between the modalities applied for the trace elements As, Pb, and Zn. However, ANOVA reveals significant differences between the modalities for the trace metals Cd, Co, and Cu ($p < 0.05$), with the 120 mg/L organic modality showing higher levels of these metals followed by the 90 mg/L organic modality.

Table 3. Effects of nutrient solutions on yield and sanitary quality of hydroponically grown vegetables in trial 2. Legend, 60 mg/L TAN biopony; 90 mg/L TAN biopony; 120 mg/L TAN biopony; Control treatment/120 mg/L TAN Resh.

Treatments	**Yield Trial 2 (g)**	**Trace Metals (mg/kg)**					
		As	**Cd**	**Co**	**Cu**	**Pb**	**Zn**
60 mg/L bioponic	6105.7 ± 113.7 ^{a}	0.14 ± 0.13 ^{a}	0.01 ± 0.007 ^{b}	0.46 ± 0.04 ^{b}	6.7 ± 1.22 ^{b}	1.32 ± 1.37 ^{a}	39.77 ± 7.02 ^{a}
90 mg/L bioponic	5088 ± 58.32 ^{a}	0.019 ± 0.02 ^{a}	0.01 ± 0.00 ^{b}	1.7 ± 0.76 ^{a}	6.44 ± 2.89 ^{a}	1.10 ± 0.21 ^{a}	34.22 ± 7.63 ^{a}
120 mg/L bioponic	4605 ± 228.40 ^{a}	0.18 ± 0.18 ^{a}	0.00 ± 0.00 ^{b}	0.53 ± 0.02 ^{b}	13.78 ± 10.54 ^{a}	1.36 ± 0.35 ^{a}	22.42 ± 3.20 ^{a}
Control treatment/ 120 mg/L	11,221.6 ± 3051.5 ^{b}	0.054 ± 0.07 ^{a}	0.07 ± 0.01 ^{a}	0.32 ± 0.08 ^{b}	4.71 ± 0.79 ^{b}	3.27 ± 1.93 ^{a}	37.1 ± 4.84 ^{a}

Treatments with at least one common letter do not have a significant difference.

4. Discussion

4.1. Dynamics of pH, EC, and NPK in Nutrient Solutions during Anaerobic and Aerobic Digestion of Chicken Droppings

Numerous studies have examined the importance of chicken droppings in the production of liquid fertilizers, particularly in organic hydroponic cultivation [58–60]. These chicken droppings are added to water in aerobic or anaerobic conditions, allowing them to ferment for one to two weeks or more, generating a digestate [61]. According to previous research, the use of highly diluted organic digestates in hydroponic cultures has yielded results similar to those of a mineral nutrient solution [62]. Conversely, nutrient solutions with a high concentration of digestate were detrimental to plants due to the high NH_4^+ concentrations, as mineral nitrogen is present in anaerobic conditions. When digestates are highly concentrated in nutrient solutions, there is a massive proliferation of heterotrophic microorganisms that can disrupt nitrifying bacteria if dissolved oxygen concentrations are low [63,64]. Both trials progressively recorded significant nitrogen losses, which can be explained by the intense development of microorganisms during the aeration phase caused by residual organic matter, as shown in Figures 5 and 6. Heterotrophic bacteria consume and assimilate all the mineral elements produced during mineralization [65,66]. This explains why the more concentrated digestate modalities experienced greater nitrogen losses than the less concentrated ones.

The transformation of NH_3-N, NO_2-N, and TAN into nitrate NO_3-N in both experiments demonstrates that ammonification and nitrification processes occurred in the formulated bioponic nutrient solutions. The increase in pH of the nutrient solutions can be attributed to various biochemical processes such as ammonification (conversion of inorganic nitrogen into ammonium ions NH_4^+, which absorb H^+), the removal of CO_2 resulting from the transformation of carbonate ions (CO_3^{2-}) and protons H+ into CO_2 and H_2O, and the removal of fatty acids [67,68]. However, under aerobic conditions, the processes of ammonification and mineralization of organic matter are significantly faster, whereas under anaerobic conditions these processes are slowed down. Therefore, in an aerobic environment, various heterotrophic microorganisms play a role in the decomposition of organic matter [65,66,69]. On the other hand, the pH of the nutrient solutions can decrease due to the nitrification process where H^+ ions are released. Conversely, the pH of the nutrient solutions can decrease due to the nitrification process where H^+ ions are released. Consequently, aerobic heterotrophic microorganisms can utilize these ions during the oxidation of organic matter, leading to the release of dissolved CO_2 in the water, which forms carbonic acid and can lower the pH of the nutrient solution [70,71]. Nearly 80% of the anions and cations absorbed by plants come from nitrogen (NH_3-N and NO_3-N). These different forms are responsible for the increase and/or decrease in pH of the medium via plant roots when the cation/anion ratio is greater or smaller than one, respectively. The results of this study showed that the pH of the nutrient solution remained near constant throughout the chicken manure digestion process. This could be explained on one hand by the fact that low levels of ammonia could lead to acidification of the plant growth medium, but on the other hand, the fact that the cation/anion ratio would be greater than one implies that an excess of cations in the medium can interfere with the absorption or availability of essential anions for plant growth, thus disrupting the chemical balance of elements in the medium [72]. Additionally, some microorganisms present in the environment (*Escherichia coli*, *Saccharomyces cerevisiae*) can produce organic acids or release hydrogen ions during their metabolism, capable of acidifying the medium leading to the unavailability of certain nutrients. Moreover, ammonia nitrogen in solution can be present as free NH_3 at alkaline pH, which could pose significant risks of nitrogen loss through volatilization into the atmosphere by reducing their concentrations [70,72–80].

In hydroponics, measuring the electrical conductivity of a nutrient solution indicates the approximate amount of mineral salts available in the solution. The total amount of ions in the solution exerts osmotic pressure on plant roots and therefore determines plant development, growth, and productivity [81]. The results obtained show that as the days of mineralization increase, the electrical conductivity simultaneously increases from 2000 to over 5000 (µS/cm), which could be explained by the fact that the chicken droppings used were naturally very rich in nutrients. Similar observations have shown that chicken manure from industrial farming with balanced poultry feed had higher electrical conductivity than other animal manures or manure from traditional farming [82,83].

4.2. Effects of Nutrient Solutions on Plant Growth in Bioponic Cultures

In both trials, visual observation revealed that the growth of lettuce plants in the bioponic treatments was marked by a delay compared to the chemical reference treatment seven days after lettuce transplantation into the rafts. This delay in plant growth could be explained by the fact that plants need to acclimate to their new growing environment, transitioning from tap water to an organic nutrient solution [84].

However, a decrease in the levels of essential elements in the organic nutrient solutions was observed. This phenomenon could be explained by the fact that treatments with the highest concentrations of dry matter and TAN contained, on one hand, large amounts of residual organic matter, and on the other hand, they developed an intense microbial activity that would disrupt the proper functioning of nitrifiers, thus making oxygen increasingly scarce in the environment.

Consequently, essential minerals released during mineralization are often consumed by heterotrophic bacteria but also assimilated by plant roots [66,85]. However, organic treatments with higher concentrations of dry matter or TAN were prone to significant nitrogen losses compared to less concentrated treatments due to the formation of biofilms on the surfaces of production systems on one hand [86]. On the other hand, these biofilms may present risks by trapping or adsorbing minerals through the formation of anaerobic zones, thus leading to the denitrification process at the expense of organic matter nitrification [66,87,88]. However, the absorption of nutrients by plants as well as the conversion of nitrites to nitrates by microorganisms can also reduce nutrient concentrations in the nutrient solution [89,90].

Phosphorus can exist in several forms depending on the pH of the medium, and its root uptake can occur via PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^{-}$ ions, with the latter two forms being the most absorbed by plants. Phosphorus is more available to plants at slightly acidic pH levels (around 5) in conditions where plants are grown on inert substrates. However, when the pH of the nutrient solution becomes alkaline or very acidic, phosphorus availability decreases [91,92]. Our results showed that the pH of the nutrient solutions was alkaline during the cultivation period, while the phosphorus concentration increasingly decreased. This phenomenon could be explained by the fact that phosphorus precipitated as calcium phosphate, lead phosphate, or magnesium phosphate, forms that are less available to plants [93–96].

4.3. Yield and Health Quality of Lettuce Plants Grown in Bioponic Cultures

Hydroponic cultivation offers several advantages, including water economy and agricultural product quality. Additionally, it provides higher crop yields compared to conventional agricultural production techniques [97–104]. In both trials, the highest lettuce crop yields were obtained with the mineral nutrient solution modality, followed by the bioponic modality with low D.M. (in the case of trial 1) and low TAN content (in trial 2). Although not statistically significantly different, great differences are observed between the modality with low D.M. and low TAN compared to the two other bioponic modalities. This is explained by the fact the plant's need for nitrogen comes mainly from nitrate and to a smaller extent from TAN. The nitrogen uptake originating from TAN remains, however, small as this one can become toxic at high concentrations [105–110]. At the beginning of the lettuce growth stage (day 21 in Figures 5 and 6), high nitrate concentrations and low TAN concentrations are found for the reference solution, which, therefore, leads to optimal growth and fine high lettuce yields. In trial 1, reference treatment and bioponic solution with the lowest D.M. (i.e., 0.35% D.M.) display comparable TAN concentrations. However, this bioponic modality does not display similar NO_3-N concentrations to the reference. In other words, the initial TAN concentration for this low D.M. modality does not limit plant growth; however, NO_3-N concentrations remain low, and growth is not particularly promoted either. Although the difference in NO_3-N and TAN concentrations existing between the reference and the bioponic solutions become smaller towards the end of cultivation in both trials, it is the difference existing at the beginning of cultivation that will have the greatest impact on plant growth and impact yield. Altogether it can be said that even if bioponic solutions do not offer the same lettuce yields, the use of low D.M. and low TAN contents can offer better yields than other bioponic options for local populations wishing to use bioponics.

Additionally, lettuce plants grown through bioponic cultivation in the agro-environmental conditions of Lubumbashi pose no risks for human consumption, with metal concentrations detected in lettuce leaves being below the toxicity threshold set by the WHO/FAO for human vegetable consumption. In regions with high heavy metal concentrations, the WHO suggests that vegetables intended for human consumption should not exceed toxicity thresholds, which are set for most trace elements, notably 10–20 mg/kg Cu, 1–5 mg/kg Co, 5–10 mg/kg Pb, and 1–2 mg/kg Cd; beyond these toxicity thresholds, vegetables containing higher levels of trace metal elements are considered contaminated. Bioponics may serve as an alternative for producing quality vegetables in an environment impacted by anthropic activities, particularly mining and mineral [103,104]. In the context of environmental contamination and pollution in the city of Lubumbashi, the use of new technologies such as bioponics may prove to be a more efficient solution to produce quality vegetables. The use of organic fertilizers in hydroponics offers numerous economic, ecological, and environmental advantages [7,111,112]. Hydroponic crops are known to be environmentally friendly because they save water resources, consume less water, and do not use too many pesticides. One of the limits consists of using synthetic chemical fertilizers. However, we, as others [113,114] contribute to the prospect of the possibility of using organic fertilizers derived from animal dung and plant debris as a source of nutrients for the plants. The productivity of vegetable crops such as Chinese cabbage in Lubumbashi gardens remains relatively low, with average yields estimated at around 1.9 kg/m^2. Additionally, harvest products from this conventional soil-based agriculture remain of poor sanitary quality. Harvest products from this new soilless technique are free from any metallic contamination and produce greater yields compared to soil-grown cultures [115].

5. Conclusions

This study aimed to develop and optimize a new bioponic technique to produce liquid fertilizer from chicken manure and implement it in the organic hydroponic (bioponic) cultivation of lettuce (*Lactuca sativa rz*). To achieve this, two types of trials were conducted under shade netting in the ambient conditions of Lubumbashi. Overall, the results are particularly promising as they demonstrate that quality vegetables can be produced, with interesting yields, exclusively using animal waste as fertilizing material. Our technique, which involves fermenting chicken droppings for hydroponic lettuce production in an environment contaminated by trace metals in Lubumbashi, has proven to be an ecological, and practically implementable approach. The results suggested that using low percentages of dry matter from chicken manure (0.35% D.M) and low concentrations of total ammoniacal nitrogen (TAN) (60 mg/L of TAN) yielded higher outputs compared to bioponic treatments receiving high concentrations of dry matter and/or TAN, respectively, for trials 1 and 2. Although yields obtained with chemical nutrient solutions remain superior to bioponic treatments in vegetable cultivation, the obtained results still demonstrate that chicken manure presents significant potential in urban agriculture.

Additionally, lettuces grown using bioponics are safe for consumption, as they contain no trace metal levels above the FAO/WHO toxicity threshold for vegetables. However, further studies should investigate nitrogen loss mechanisms in the rafts, the role of nitrifying bacteria in organic matter, and the valorization of methane gas produced during the anaerobic fermentation process of chicken manure. Lastly, additional studies could test the cultivation of local plant species with added value and other types of organic matter in bioponic vegetable cultivation.

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Appendix A

Table A1. Physico-chemical characteristics of chicken droppings.

	Essential Elements		Heavy Metals (mg/Kg)					
	Mg (%)	**Ca (%)**	**Cu**	**Co**	**Cd**	**Pb**	**Zn**	**Fe**
Chicken droppings	0.11	7.48	80.5	5.6	0.04	0.3	321.3	654

Table A2. Physico-chemical properties of bioponic nutrient solutions from trial 1, stock solutions before dilution in rafts on day 7, after dilution on day 21 of vacuum aerobic circulation pH; EC: electroconductivity (µS/cm); TAN: total ammonia nitrogen (mg/L); NO_3-N: nitrate nitrogen (mg/L); NO_2-N: nitrite nitrogen (mg/L); TMN: total mineral nitrogen (sum of NO_3-N, NO_2-N, and TAN) (mg/L); PO_4^{3-}: phosphate-phosphorus (mg/L) and K-potassium (mg/L). Legend, T1: 0.35% dry matter; T2: 3.5% dry matter; T3: 7% dry matter.

Parameters	**Stock Solutions (mg/kg)**			**Solution after Aerobic Digestion (mg/L)**					
				T1		**T2**		**T3**	
	T1	**T2**	**T3**	**7**	**21**	**7**	**21**	**7**	**21**
pH	6.9	6.9	6.7	7.4	7.2	7.4	7.4	7.3	7.3
EC	726.5	795.3	747.3	546	591.4	654	696.8	944	959.4
TAN	225.06	994.26	2112	25.4	3.72	30.83	19.17	89.13	37.12
NO_2-N	4.3	28	84.6	0	0	0	1.33	0	2.33
NO_3-N	3.4	61.6	472	3.53	7.07	0.83	2.83	4.13	4.2
TMN	232.8	1083.8	2668.6	29	10.78	31.67	23.33	93.27	43.65
PO_4^{3-}	37.3	49	184	51.7	67	13	62.33	22	40.33
K	81.6	333.3	1666.6	8.8	8.83	12	20.17	21.17	13.67

Table A3. Physico-chemical properties of bioponic nutrient solutions from trial 2, stock solutions before dilution in rafts on day 7, after dilution on day 21 of vacuum aerobic circulation pH; EC: electroconductivity (µS/cm); TAN: total ammonia nitrogen (mg/L); NO_3-N: nitric nitrogen (mg/L); NO_2-N: nitrite nitrogen (mg/L); TMN: total mineral nitrogen (sum of NO_3-N, NO_2-N, and TAN) (mg/L); PO_4^{3-}: phosphate-phosphorus (mg/L) and K: potassium (mg/L). Legend, T1: 60 mg/L TAN biopony; T2: 90 mg/L TAN biopony; T3: 120 mg/L TAN biopony.

Parameters	**Stock Solution**	**Solution after Aerobic Digestion (mg/L)**					
		T1		**T2**		**T3**	
		7	**21**	**7**	**21**	**7**	**21**
pH	6.4	7.7	7.5	8	7.5	7.9	7.9
EC	3818.8	508	611	579.1	837.6	873	1125.3
TAN	960.8	5.37 ± 1.52	1.60 ± 0.34	5.60 ± 0.34	48.20 ± 33.33	8.04 ± 1.78	124.67 ± 34.82
NO_2-N	114.4	30 ± 16.3	211.6 ± 20.95	10.00 ± 1.41	250 ± 96.26	25.00 ± 2.16	110 ± 57.15
NO_3-N	301.1	18.0 ± 4.81	36.67 ± 7.41	52.83 ± 20.09	40.67 ± 9.88	24.07 ± 4.65	40.50 ± 29.15
TMN	1376.4	53.37 ± 19.84	249.93 ± 13.80	68.43 ± 19.50	338.86 ± 63.62	57.10 ± 5.52	275.17 ± 51.25
PO_4^{3-}	182.4	38.23 ± 12.5	77 ± 20.51	60.6 ± 18.95	43.33 ± 21.93	86.33 ± 19.07	48.00 ± 9.09
K	240.4	7.23 ± 3.51	31.33 ± 5.56	13.03 ± 4.58	49.00 ± 5.35	11.20 ± 5.37	56.83 ± 17.46

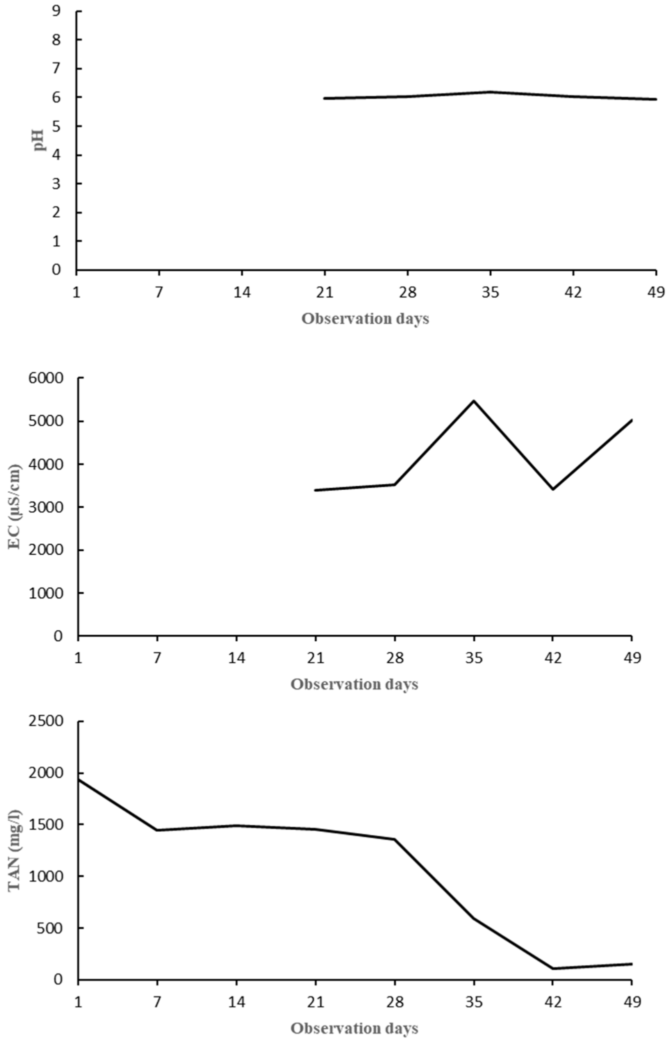

Figure A1. Effect of dry matter on the quality of nutrient solutions derived from chicken manure in trial 2.

Figure A2. Illustration of lettuce sowing in rockwool cubes and cultivation.

Figure A3. Illustration of bioponic lettuces produced in an impacted environment in Lubumbashi.

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