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Analysis of lettuce (*Lactuca sativa*) production in different substrates in an aquaponic system using an IBC container

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Abstract**—***Aquaponics is amethod of food production that integrates two types of cultivation in one unique place. Fishes and plants can be cultivated in this system, where the fish are maintained in recirculating aquaculture system and the plants in hydroponics (cultivation of vegetables without soil). In order to demonstrate that aquaponics is an alternative for the cultivation of vegetables and also a possibility of water treatment, the objective of the present study was to evaluate the water quality of an aquaponic system composed by lettuces (*Lactuca sativa*) and lambari fishes (*Astyanax bimaculatus*). Additionally, we evaluated which is the best civil construction residue (gravel, brick and expanded clay) for lettuce cultivation. The microbiological and physical - chemical monitoring of the system was carried for 42 days, and water from the fish tank was analyzed every 15 days, in the decanter, in the sump tank and in the cultivation bed. The microbiological analyzes consisted in total and thermotolerant coliforms and heterotrophic mesophilic count. For physical – chemical parameters, we analyzed pH, Dissolved oxygen (DO), Toxic Ammonia and Nitrite. Additionally, the development of lettuce in the three different civil construction residues was determined by the average total weight, average leaf mass and average root mass of the plants. We concluded that there was total and thermotolerant coliforms in all the stages of the collection. There was a better growth of the lettuce in the substrate of broken brick, followed by expanded clay and finally by crushed stone.*

Keywords**—Aquaponics, Astyanax bimaculatus, Civil construction residue, Sustainability.**

I. INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) defines aquaculture as the cultivation of aquatic organisms including crustaceans, fish and molluscs. Such activity can be developed in both fresh and salt water, but it must always be under controlled conditions. This type of farming is currently one of the most important forms of production, being responsible for half of fish and seafood consumed by the world population (SEBRAE, 2015). Hydroponics, on the other hand, is the cultivation of plants without the use of soil, in which the necessary nutrients for plant growth are provided through an aqueous solution (BEZERRA NETO, 2017).

The union of the Aquaculture x Hydroponics activities results in a new technology named Aquaponics. A modality of food production with low water consumption and high utilization of the organic waste generated (CARNEIRO et al., 2015). Such activity, owing to characteristics of sustainability can be framed as a solution for traditional methods of agriculture, which shows high environmental impacts (HUNDLEY, 2013).

Water recirculation systems provide a substantial advantage by drastically reducing the required area for aquatic organisms. Despite this, the high densities of storage normally present a disadvantage related to

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production of large volumes of organic waste, which must be removed (CARNEIRO et al., 2015; PINTO, 2015).

According to EMBRAPA (2018), aquaponics can reach 90% economy of water regarding conventional agriculture, due water utilization be cyclical. Because it is an integrated culture where later stages benefit from previous stages, aquaponics presents a series of benefits for itself and the environment. Hundley (2013) comments that with increasing restrictions on water use and costs, rural producers have been looking for cheaper and more efficient alternatives for food production.

In conventional aquaculture, all excretions from aquatic organisms remain in the water, causing their accumulation and increasing the toxicity of the environment. In aquaponics, with the presence of bacteria that fix nitrogen, the fish excrement will be transformed in nitrate and nitrite, assimilable by plants (SEBRAE, 2015).

The success of aquaponics depends on the quality of water supply, quality and quantity of the food supplied, the residence time of the effluent within the systems, the selected species, the stocking density and the biomass of the organisms (OAK et al., 2017). According to Tonet et al. (2011), one of the precautions that must be taken in relation to aquaponics is microbiological contamination, since fish excrements are used as nutrients, which can contaminate water and vegetables.

According to Ferreira (2013) aquaponics is a biosystem of food production that fits as a polyculture, since the waste produced in one of the biological systems is consumed in a second. As a result, all waste is minimized, making the production able to be self-sustainable, allowing healthy, sustainable and profitable production to reach large urban centers.

The objective of this study was to evaluate the water quality of an aquaponics system composed by lettuces (*Lactuca sativa*) andlambari fishes (*Astyanax bimaculatus*), and to evaluate which is the best civil construction residue (crushed stone, brick and expanded clay) for lettuce cultivation. Our results may assist in the development of cultivation protocols that reduce water consumption during food production, aiming to reduce the impacts that this production causes to the environment.

II. MATERIALS AND METHODS

2.1 Aquaponics System

The Aquaponics pilot system, built at the Institute of Technological and Exact Sciences (ICTE) for the Scientific Initiation Research of Stefan Cardoso and Pedro Bianchini (CARDOSO et al., 2019) was used. The entire system was adapted based on a review of the EMBRAPA (Brazilian Agricultural Research Corporation) literature. Figures 1 - 4 in the next topic, represents the scheme of the system.

In this project, three types of beds from different cultures were used: the first bed was made of crushed stone, the second of broken brick and the third of expanded clay. These selected substratesare waste from the civil area and are generally discarded in the environment.

2.2 Microbiological analysis of the water in the fish tank, sump and rhizospheric filter

Four samples of water from the aquaponic system were analyzed with collections carried out every two weeks, all taken at 08:00a.m. Brasília time, totaling 42 days of sampling. Such samples were taken from the fish tank, decanter, sump and the culture bed. The water was collected in sterile bags, so as not to interfere with the results.

Analyzes of total coliforms (37º C), thermotolerant coliforms (45º C) and Heterotrophic Mesophilic Count were performed. They all were done in duplicate according to the methodologies described by Silva et al. (2010).

For water, the count of total and thermotolerant coliforms wasperformed using the Most Likely Technique. Five tubes with a Durhan tube containing Lauryl Sulfate Tryptose broth (LST) were used. Later, after the samples were positive, they were incubated in a tube with Bright Bile Green at 37º C and medium EC broth (EC) at 45º C for 24-48 hours.

The counting of heterotrophic mesophiles was done using the Pour Plate technique. Three serial water dilutions were prepared, and one ml of each dilution was placed into a sterile Petri dish. Approximately 20 ml of Plate Count Agar agar was added. The plates were incubated at 37º C for 48 hours.

2.3 Physical-chemical analysis

The analyzes were conducted according to the official methodology of FUNASA (National Health Foundation, 2006), as follows:

2.3.1 pH

The phmeter was used. The device was turned on and it was expected to stabilize. The device was calibrated with standard solutions (pH 4 and 7), then the electrode was introduced into the sample to be examined and read.

2.3.2 Dissolved Oxygen (DO) and Temperature (in the field)

For the determination of DO and temperature, a digital oximeter of the brand Hach (model HQ40d) was used. The probe was immersed in the collection points of the

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aquaponic system (fish tank, decanter, sump and rhizospheric filter outlet).

2.3.3 Toxic ammonia

To evaluate the toxic ammonia present in the system, a colorimetric test was used from the LABCONTest brand. For each sample of the system, 8 drops of reagent solution 1 (Phenol, sodium nitroprusside, isopropyl alcohol and distilled water) were dripped, the beaker was capped and stirred. Then 4 drops of reagent solution 2 (sodium hydroxide, sodium hypochlorite and distilled water) were dripped, the beaker was capped and stirred.

After three minutes (time for reaction), the reading was done.

2.3.4 Nitrite

To evaluate the presence of nitrite in the system, a colorimetric test of the LABCONTest brand was used. For each sample of the system, 2 drops of reagent solution 1 (sulfanilic acid, acetic acid and distilled water) were dripped, the beaker was capped and stirred. Then 2 drops of reagent solution 2 (Alpha-naphthylamine and ethyl alcohol) were dripped, the beaker was capped and stirred. After ten minutes (time for reaction), the reading was done.

2.4 Analysis of lettuce growth

Lettuce (*Lactuca sativa*) productivity was evaluated as follows: for each different substrate, three plants of lettuce were randomly chosen. The plants were weighed using a semi-analytical balance, where the average total weight (ATW), average leaf mass (ALM) and average root mass (ARM) were determined.

III. RESULTS AND DISCUSSION

3.1 Aquaponics Pilot System

The first barrel, with decanter function, receives water from the fish tank through an entrance on the bottom. The barrel has a round shape, and because of this, it was installed in the entrance of water one 90º elbow. In this way, the water can enter tangentiating the wall of the recipient, creating a circular movement inside (Figure 1 and 4). This movement is responsible to the process of decantation where the solids are retained in the bottom (CARDOSO et al., 2019).

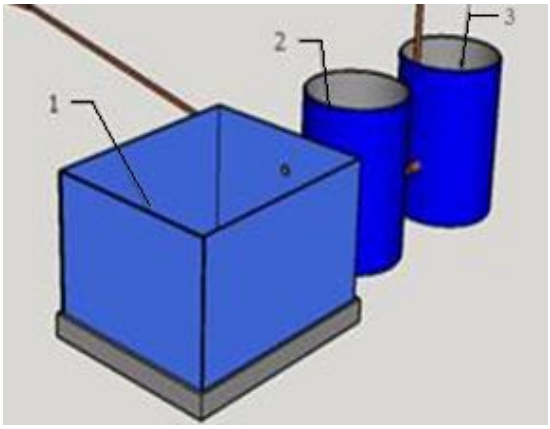

Fig. 1: Disposal of tank and barrels. 1. Fish tank; 2. Decanter; 3. Sump tank

As well as the grow bed, which has the main function of rhizospheric filtration, three similar structures with smaller size and volume were built. These structures had the same functioning of the first, but with different objectives. Because the first structure is part of the filtration process, *Xanthosoma sagittifolium* (taioba) was planted. In the other three, *Lactuca sativa* (Lettuce) was planted (Figures 2 and 3) (CARDOSO et al., 2019).

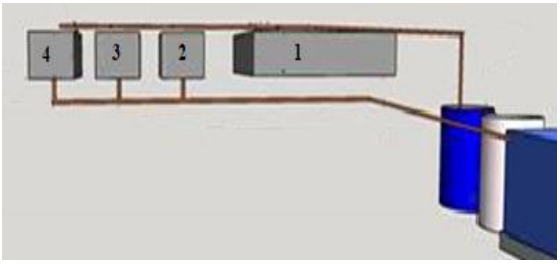

Fig. 2: Disposition of the lettuce grow beds. 1. Rhizospheric filterwith Xanthosoma sagittifolium*; 2.Grow bed with stone gravel; 3. Grow bed with expanded clay; 4. Grow bed with broken bricks.*

Fig. 3: Disposition of the lettuce grow beds and rhizospheric filter.

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Fig. 4: Disposition of fish tank, decanter and sump tank.

3.2 Water microbiological analysis

In the interval of 15 days of water collection in the four tanks, there was an increase in the Standard Count in plate, i.e. an increase of the heterotrophic mesophilic microorganisms in the water. This was already expected, because there was an increase in the size of the fish, thus increasing the amount of feces and probably the formation of biofilms in the tanks(Figure 5).

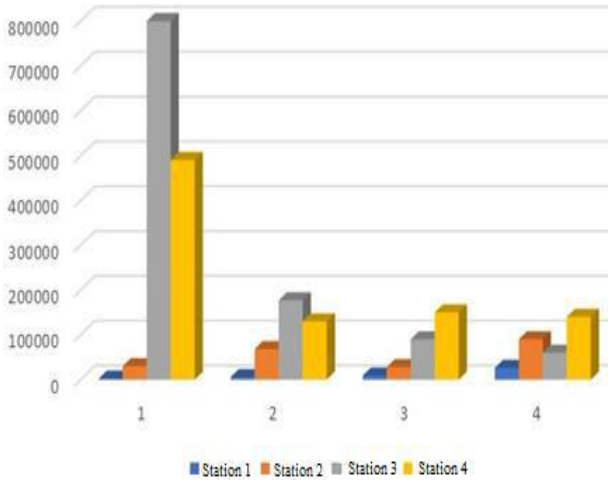

Fig. 5: Standard Count plate results in the water collected in the fish tank (1), in the decanter (2), in the sump tank (3) and in grow bed (4) of four collects.

Total and thermotolerant coliforms counted in all four tanks resulted in amounts of > 1.600 MPN / ml (Most Problably Number / ml) of analyzed water, in all collected samples. In the aquaponics process, the water used contains leftover food and fish waste, which are used as a nutrient for plants, thus its coliform contamination is more evident.

These results were compatible with those found by Tonet et al. (2011), where 100% of their samples showed contamination by total and thermotolerant coliforms in aquaponics cultivation water.

3.3 Physical-chemical analysis

3.3.1 pH

Potential for Hydrogen (pH) is one of the critical parameters in an aquaponics system. This is because it has at least three distinct organisms (fish, plants and bacteria) in the same system. According to EMBRAPA (2015), nitrifying bacteria have an optimum pH in the range of 7.0 to 8.0. In contrast, in hydroponics, plants show their greatest growth in pH ranges from 5.5 to 6.5. For lambari fishes (*Astyanax bimaculatus*) grown in aquaponics, pH is in the range of 7.0 to 9.0.

Through the data obtained (Figure 6), it is possible to determine that, in the experimental system, bacteria and fish were subjected to excellent conditions, but the ideal range for plants was not reached. Thevalues were similar to results obtained by Kuhnen et al. (2016), who found pH in the range of 7.5 to 7.9.

The pH value = 6.5 has an acid character, which is beneficial for the plant, because when the pH of the substrate is basic, the ions would precipitate and the plants would not be able to absorb them. The pH in the present system has not been manipuled, but we believe that a reduction could lead to improvements in the aquaponic production as a whole.

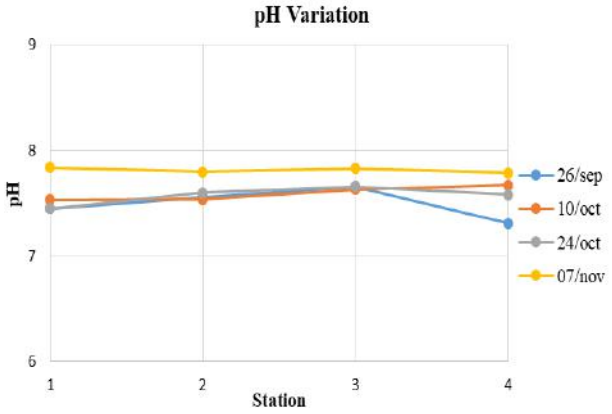

Fig. 6: Results of variation of pH in the water collected in the fish tank (1), in the decanter (2), in the sump tank (3) and in grow bed (4) of four collects.

3.3.2 Dissolved Oxygen (DO)

The dissolved oxygen values increased gradually and following a pattern in each study Station (Figure 7). For Stations 1 and 2 the values are practically identical because in the fish tank (Station 1), although there was a good oxygenation mechanism, the breathing of fish consumed oxygen. For the decanter there was no oxygenation mechanism.

In Station 3 the fall of water from Station 2 provided a destabilization on the surface, providing the insertion of

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DO by diffusion, thus explaining the highest concentrations. The lastStation, related to the cultivation bed (Station 4), had its OD levels lower than in the previous Station, probably due to consumption by nitrifying bacteria. The DO concentration over time in each Station has increased, which is beneficial for the aquaponic system. The dissolved oxygen values were higher than 5mg/L, which isrequired by the CONAMA (National Environment Council) resolution. Values above 5mg/L allow adequate aeration for fish (BARBOSA, 2011)

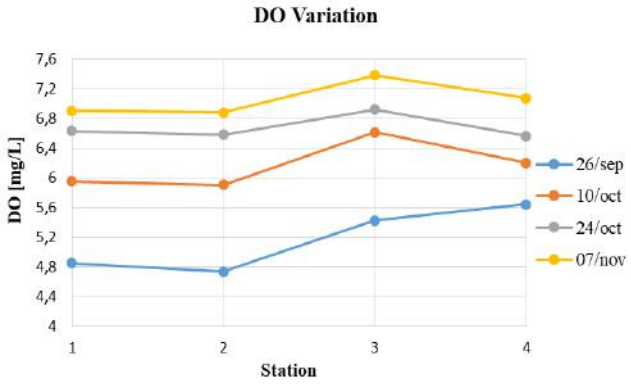

Fig. 7: Dissolved oxygen (DO) in fish tank (1), decanter (2), sump tank (3) and grow bed (4) of four collects.

According to EMBRAPA (2018), for aquaponics in a tropical climate, the amount of DO in water must always be higher than 3 mg/L. This parameter is monitored looking for the welfare of the organisms that inhabit that environment. During our study, the values obtained were always above the minimum concentration cited.

3.3.3 Temperature

Jordan (2011) defines the ideal temperature of 26ºC for an aquaponics system. This temperature favors the fattening of fish and meets the requirements of other organisms present in the system. During our study, the recommended temperatures were reached only in the last week of experiments (Figure 8).

In the absence of an equipment that could control this parameter, the experiment had the local climate as a determining factor. However, as all measurements were made in the morning, these temperatures increased during the day.

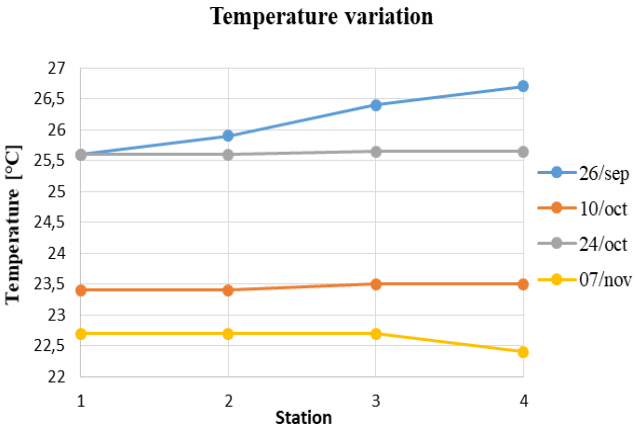

Fig. 8: Temperature variation in fish tank (1), decanter (2), sump tank (3) and grow bed (4) of four collects.

INMET (National Institute of Meteorology) was consulted with the maximum and minimum temperature data of the station located at UFTM - ICTE 2. This station was used because it is located very close to the experiment and reliably represents the climatic conditions of the study region. The temperatures were organized in Table 1 and show that the measurements were performed accurately, as they are within the Minimum and Maximum interval on the day of each measurement.

Table 1. Variation oftemperature(T) of the region in which the experiments were conducted. Minimum (min) and maximum (max) values.Data from INMET (National Institute of Meteorology), UFTM, ICTE2.

Date	T min [°C]	T max [°C]
26/09/2018	20.5	34.3
10/10/2018	19.5	30.7
24/10/2018	20	26.7
07/11/2018	20.4	27.4

3.3.4 Ammonia and nitrite analysis

Nitrification is a chemical-biological process that allows the formation of nitrate through the metabolism of nitrifying chemosynthetic bacteria. In this process, ammonia (present in fish excreta) is converted into nitrate. When released, nitrates are available for assimilation by plants. This cycle is divided into three stages, nitration, nitration and nitrification (RIBEIRO, 2016).

The concentration profile of toxic ammonia and nitrite followed the expected pattern, starting with a high value and showing a reduction over time. This reduction highlights the absence of toxic ammonia in post-filtering steps (Station 4) (Figures 9 and 10). According to EMBRAPA (2015) the levels tend to stabilize when the colonies of bacteria are formed and mature.

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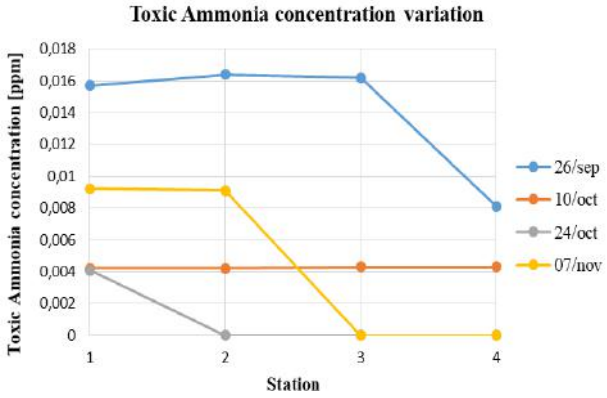

Fig. 9: Toxic ammonia concentration in fish tank (1), decanter (2), sump tank (3) and grow bed (4) of four collects.

The Stations with the highest concentrations of these two parameters (Figure 8) were the fish tank and decanter, respectively. All levels of toxic ammonia and nitrite concentration were in accordance with the reference values established by the company providing the colorimetric tests, featuring an efficient filtering process and a favorable and pleasant environment for the fish.

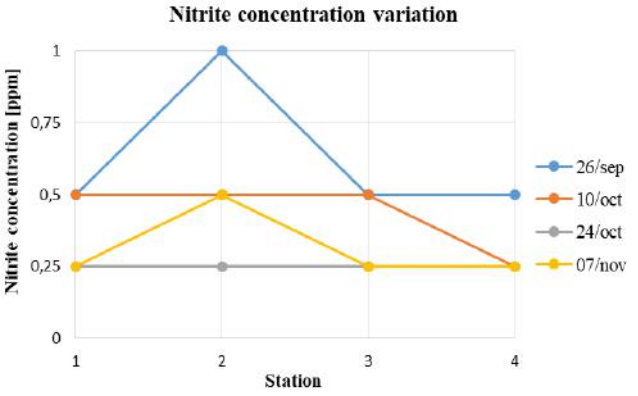

Fig. 10: Nitrite concentration in fish tank (1), decanter (2), sump tank (3) and grow bed (4) of four collects.

Graber and Junge (2008) point out that there are ways to optimize nitrification, degradation, denitrification and absorption in this type of system, maximizing production. These processes occur with the degradation of the organic matter excreted by the fish, by the bacteria of the biological filter.

3.4 Analysis of lettuce growth

The grow bed containing broken brick was the one that showed the greatest growth of lettuces, with a total average weight of 74.49g. The grow bed containing expanded clay, obtained an intermediate growth with a total average weight of 30.70g. For crushed stone the total average weight was 17.28g (Figure 11).

Fig. 11: Lettuces in the respective substrates. A. Broken brick; B.Expanded clay; C.Crushed stone.

The results for lettuce leaves followed the previous pattern in relation to different substrates, i.e. 62.52g,24.62g and 14.13g for broken brick, expanded clay and crushed stone, respectively(Figure 12).

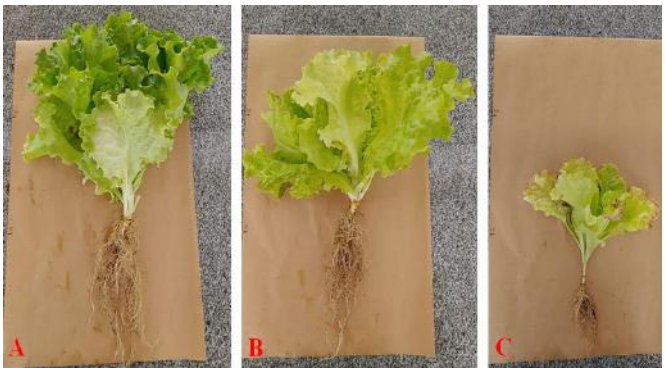

Fig. 12: Comparison of leaf growth of lettuces.A. Broken brick; B.Expanded clay; C.Crushed stone.

The results of root growth also followed the pattern of total average weight and leaf growth, i.e. 11.97g for broken brick, 6.08g for expanded clay, and 3.15g for crushed stone (Figure 13).

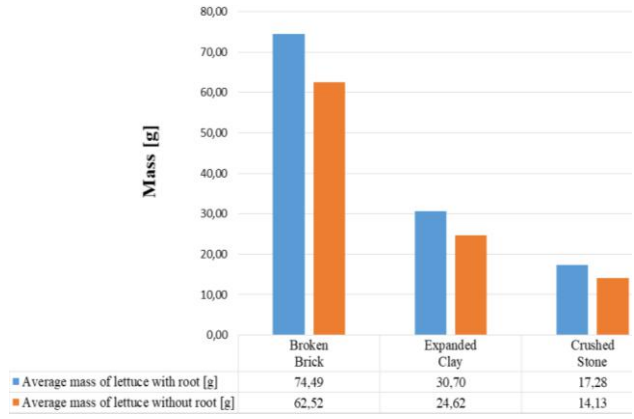

Fig. 13: Comparison of root growth of lettuces in different substrates.

The best substrate for the cultivation of lettuce was substrate A. The brick fragments are composed of an extremely porous material, which houses greater colonies of nitrifying bacteria. This converts the ammonia into nitrite and later into nitrate, so that the plants can assimilate. This probably contributed to the enablement of the further development of lettuces. The substrate

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containing crushed stone provided the lowest satisfactory results. This is probably because the gravel is not porous, as explained above.

IV. CONCLUSION

For lettuce (*Lactuca sativa*) growth, better results were obtained in the substrate of broken bricks, expanded clay and crushed stone, respectively. In the substrate of broken bricks, lettuce presented, in addition to a higher growth, a better visual aspect of the leaves.

For the removal of ammonia, the system proved to be extremely efficient, since the highest concentrations were obtained at the beginning of the experiment and over the time these concentrations turned into zero. We can conclude that the filtration system matured during the time of the experiment until reaching its optimum efficiency.

We observed that total coliforms and thermotolerant coliforms were present in all stages of aquaponics, indicating that hygiene procedures before the consumption of vegetables must beperformed, regardless of the cultivation technique.

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