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MICROBIOLOGICAL QUALITY OF LETTUCE IRRIGATED WITH TREATED WASTEWATER

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ABSTRACT

It is necessary to search for alternative sources of water where the use of potable water is not required. The reuse of treated domestic effluent is an alternative for crop irrigation. This research was conducted using a complete randomized block design in a 3 x 3 factorial scheme with five replications, in a protected environment at the Federal University of Sergipe. This study was designed to evaluate the microbiological quality of *Baba de Verão* (*Lactuca sativa*) irrigated with different concentrations of effluent (0, 50 and 100%) in two cycles and three irrigation depths, corresponding to 75, 100 and 125% field capacity. The leaves were submitted to microbiological analysis to verify if they were contaminated by treated wastewater used for irrigation of the crop. The analyzed variables were thermotolerant coliforms and Salmonella. The microbiological quality of lettuce was classified according to the standards required by the National Sanitary Surveillance Agency for the consumption of fresh vegetables. Treated domestic wastewater can be considered a possible source of water for lettuce when water is applied to the soil in proximity to the root system of the crop.

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INTRODUCTION

Due to an increasing world population, associated with the capitalist economic system, water sources are being degraded. Although, located in a country with high per capita water availability, the Brazilian Northeast, situated in arid and semi-arid regions, has long periods of drought with a strong solar incidence, which increases evaporation and reduces the amount of accessible water. Therefore, water is a limiting factor for development in this region. Agricultural production with the use of agrochemicals, irrigation and the direct release of domestic effluents, responsible for contamination with thermotolerant coliforms and other bacteria in water bodies are aggravating factors for the degradation of water resources (Nonga *et al*. 2011; Hachich, 2012). Campos *et al*. (2015) state that water scarcity in arid and semi-arid regions has become increasingly recurrent, especially in developing countries and rural areas. Silva *et al*. (2014) argue that the use of wastewater in agriculture is a valid alternative, since it provides the necessary water for the increase of agricultural production, as well as reducing the demand pressures in the springs. Resolution 121 of 2010 established guidelines and criteria for reuse in the agricultural and forestry modality and states that reuse for agricultural/forestry purposes ought not to put the environment or public health at risk (Brazil 2010). According to the World Health Organization (2006), in regions with precarious sanitation conditions and untreated wastewater are widely used in agriculture, there are a high incidence of protozoan and virus infections, in addition, intestinal worms pose health risks. Irrigated food, especially vegetables, are characterized by their importance in food and human health and its consumption has grown not only by population growth, but also by the tendency to change in the consumer eating habits (Allydice-Francis, Brown, 2012). Lettuce (*Lactuca sativa*) originates from wild species, which in present times are still found in southern Europe and Western Asia. It is an annual plant which belongs to the family Asteraceae (*Compositae*) and is an herbaceous, thus possessing flexible stems (non-woody); in addition, its leaves can be smooth or curly, forming or not a "head" (Filgueira 2007). According to Silva *et al*. (2011), lettuce is an important source of minerals and vitamins, besides being the hardwood crop most consumed by Brazilians. These characteristics make lettuce a food of extreme relevance for human health. The state of Sergipe produces enough lettuce to serve local commerce and the municipality of *Itabaiana* stands out as the largest vegetable producer in the state.Its central geographic location and the large fleet of trucks facilitate the distribution of production to cities and to neighboring states (Silva 2011). In this context, the objective of this work is to observe the effects of treated domestic effluent on the microbiological quality of the leaves of *Baba de Verão* (*Lactuca sativa*), and at the end of the experiment, the possibility of replacing the best quality water for an inferior source without contaminating the plant material.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse, located in the Department of Agronomic Engineering at the Federal University of Sergipe, located in the municipality of São Cristóvão, Northeastern Region of Brazil and positioned in the eastern sector of the state of Sergipe. In this experiment, water from the Sergipe Sanitation Company (DESO) and wastewater from the Effluent Treatment Station atthe Federal University of Sergipe was used. The sewage from the aerobic reactor was used, which is the second tank of the Effluent Treatment Station. The lettuce variety *Baba de Verão* (*Lactuca sativa*), belonging to the group of *Asteraceae* was utilized in this experiment. The seed used was from the brand ISLA, which has, according to the manufacturer, 98% germination and 99.8% purity. Seeding was done in a tray of 150 cells, placing about 5 seeds in each of them. Irrigation was performed twice a day, at cooler times as recommended by Embrapa (2007), the first being at 7:30 a.m. and the second at 4:30 p.m. Germination occurred approximately four to five days after sowing. Transplanting occurred about twenty days after sowing, when the seedlings had four leaves, as the literature recommends. Three seedlings of the *Baba de Verão* lettuce were transplanted into each pot and after stability of the crop, which occurred seven days after transplanting, thinning was done, leaving only the most vigorous per pot, when the differentiation of treatments started. The plots were composed of three different percentages of wastewater with supply water from DESO, E0 (100% of water supply), E1 (50% of treated effluent + 50% of water supply), E2 (100% treated effluent), and three separate irrigation slides, 100% (L1), 75% (L2) and 125% (L3) soil field capacity, with five replications, in a randomized block design, generating a factorial of 3 x 3. The determination of the irrigation depth was related to moisture in the field capacity condition, totaling forty-fivepots and nine treatments. The soil used came from the Campus of the Federal University of Sergipe, which is classified as Ultisoland is characterized as deep to shallow; moderately to well drained; very variable texture, but with a predominance of average surface texture, and clayey, in the subsurface texture; and has total porosity ranging from low to medium (Embrapa 2006). Chemical analysis of the soil was carried out, verifying the need for correction, liming with the incorporation of limestone throughout all the soil in the pot, and then the saturation of the soil until water percolation was observed. A period of approximately 90 days was expected to take place for pH correction and soil fertility. Fertilizing was done at the moment of planting in all pots, and nitrogen fertilization was performed on pots with 100% water supply. Two cycles of Lettuce crop were carried out, the first one in August and the second in October 2016 (Figure 1).

Figure 1. Treatments with lettuce cultivation

The application of water to the pots was done manually by means of a graduated 100 mL beaker, avoiding contact with the lettuce. Table 1 shows the acronyms and respective descriptions of the treatments which were used.

Table 1. Description of the acronyms of each treatment

Acronyms	DESCRIPTION
E_0L_1	100% water supply; 100% field capacity
E_0L_2	100% water supply; 75% of field capacity
E_0L_3	100% water supply; 125% of field capacity
E_1L_1	50% water supply + 50% effluent; 100% field capacity
E_1L_2	50% water supply + 50% effluent; 75% field capacity
E_1L_3	50% water supply + 50% effluent; 125% field capacity
E_2L_1	100% effluent; 100% field capacity
E_2L_2	100% effluent; 75% of field capacity
E_2L_3	100% effluent; 125% of field capacity

For moisture monitoring, nine tensiometers were installed, one in each treatment, in randomly chosen pots. Irrigation was performed when the tensiometer reading indicated that the observed humidity was below the moisture in the field capacity. The required irrigation depth was applied to raise the current moisture to the moisture condition in the field capacity of the analyzed treatment. In order to know the moment of the irrigation from the tensiometers, the water content in the soil was determined under varied tensions. From this information, the retention curve was elaborated (Figure 2). The range adopted for 100% field capacity (CC) was 15 KPa and 35 kPa (critical soil moisture accounting for the height of the water column inside the tensiometer). A relationship was made to find the bands at 75 and 125% of the CC, with 25.8 - 60.23 kPa and 9.84 - 22.97 kPa, respectively. According to the Brazilian Agricultural Research Corporation (EMBRAPA, 2007), vegetables under protected cultivation have a critical stress -30 kPa.

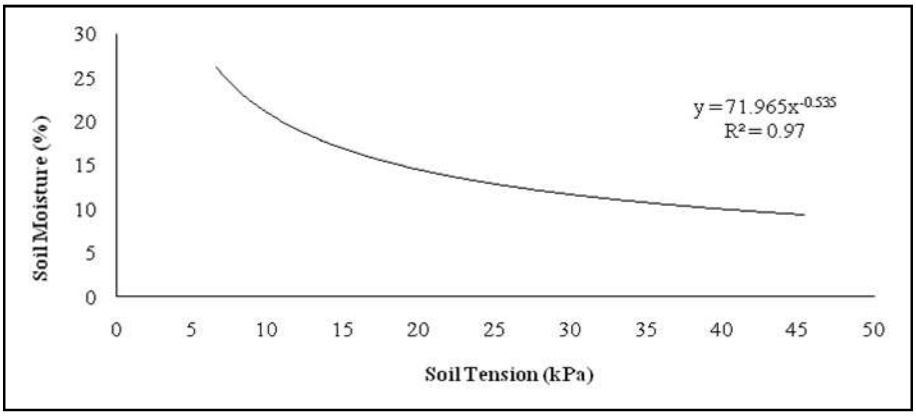

Figure 2. Water retention curve in soil to determine the relationship between soil moisture and soil tensions

Table 2. Microbiological quality of lettuce irrigated with wastewater in the two cycles

	E_0L_1	E_0L_2	E_0L_3	E_1L_1	E_1L_2	E_1L_3	E_2L_1	E_2L_2	E_2L_3
1[st]cycle									
Thermotolerant coliforms (NMP g^{-1})	< 3.0	< 3.0	< 3.0	9.3	9.3	9.3	9.3	3.6	9.2
Salmonella (in 25 g)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
2[nd]cycle									
Thermotolerant coliforms (NMP.g^{-1})	< 3.0	< 3.0	< 3.0	2.3	3.0	3.5	9.2	<3.0	<3.0
Salmonella(in 25 g)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

The leaves were submitted to microbiological analysis to verify if they were contaminated by treated wastewater used for irrigation of the crop. For this, a composite analysis of each treatment was performed, being withdrawn from each repetition two to four leaves. Preference was given to the leaves closest to the soil, since they are more susceptible to contamination. The variables analyzed were thermotolerant coliforms and salmonella, parameters required by the Health Surveillance Agency for the consumption of fresh vegetables. For the determination of the microbiological quality, the samples were analyzed according to the Compendium of Methods for the Microbiological Examination of Foods (Downes and Ito, 2001). The analysis was performed at the Laboratory of the Technological and Research Institute of the State of Sergipe.

RESULTS AND DISCUSSION

Table 2 shows the values of thermotolerant coliforms and *Salmonella* present in the lettuce for each treatment in the first and second cycle. To verify the microbiological quality, it was followed by Resolution nº 12/2001 of the Health Surveillance Agency, which allows, in raw vegetables consumed up to 10^2 NMP.g^{-1} for thermotolerant coliforms at 45° C, or thermotolerant coliforms; and requires absence of *Salmonella*. According to this information, it was found that in all treatments, in both cycles, they are within the limit of satisfactory sanitary conditions. Urbano (2013) analyzed the health characteristics of the lettuce type Elisairrigated with wastewater treated in a drip system. Similarly, the author found values within the limits allowed by Health Surveillance Agency. Santos *et al*. (2010) investigated five areas of vegetation, which were produced for commercialization, in order to verify if they existed and were contaminated by thermotolerant coliforms and *Salmonella*. A total of 140 samples were collected from coriander, lettuce, mint and cabbage. The results showed the absence of *Salmonella*, but found values above the upper limits forthermotolerant coliforms, mainly for lettuce and coriander cultures. Carvalho *et al*. (2013) when analyzing microbiological quality of sunflower, found values within the limit required by the legislation for the two variables. Dantas *et al*. (2014) in a similar way also verified levels of thermotolerant coliforms and *Salmonella* within the limit established by the resolution, for radish culture. Juchen *et al*. (2013) analyzed the microbiological quality of lettuce irrigated with agroindustrial wastewater and verified that the levels of microorganisms in lettuce were lower than those ones established in the Brazilian Legislation. Silva *et al*. (2016) studied the quality of the lettuce irrigated with water from the reservoirs of the rural area of *Caruaru*city and verified that the coliform counts were higher than the ones allowed by Brazilian legislation, which were worse than the results obtained in this experiment wastewater from domestic treated effluents. Varallo *et al*. (2011) evaluating the sanitary quality of fertigated lettuce with domestic wastewater, via drip, obtained absence of thermotolerant coliforms in the leaves. Souza *et al*. (2013) evaluated the microbiological quality of chili fruits produced with swine waste water and verified that they were not contaminated by thermotolerant coliforms and Salmonella spp., being in agreement with the sanitary microbiological standards required by the Brazilian Legislation.

Conclusions

Microbiological analyzes found that lettuces irrigated with effluent were within the limits allowed by Resolution 12/2001 of the Health Surveillance Agency. Irrigation with wastewater is a valid option, as it has been shown to meet the nutritional and water requirements of the *Baba de Verão* lettuce, in addition to being within the standards required by the legislation.

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