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RESPONSE OF TREATED DOMESTIC EFFLUENT IRRIGATION ON MICROBIOLOGICAL CHARACTERISTICS OF BEAN GROWN IN PROTECTED ENVIRONMENT

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ABSTRACT

The use of domestic wastewater treated for irrigation may become a viable alternative for regions facing water scarcity. The objective of the present work was to evaluate the microbiological quality of bean (*Phaseolus vulgaris L.*) BRS-Pontalcultivar submitted to different irrigation depths with treated domestic effluent. The irrigation depths were obtained according to 50, 75, 100 and 125% of the crop evapotranspiration and the concentrations corresponded to 0, 50 and 100% of treated domestic effluent. The experimental design was randomized blocks, in a 3 x 4 factorial scheme with four replications, in a protected environment. The effluent was collected at the Sewage Treatment Station (STS) belonging to the Federal University of Sergipe, SE, Brazil, were the experiment was conducted. The presence of Thermotolerant coliforms (NMP g^{-1}), mesophiles (CFU g^{-1}), molds and yeasts (CFU g^{-1}), and *Salmonella* were evaluated. The results of the bean grain samples were submitted to analysis according to the parameters recommended by Resolution 12 of the National Agency of Sanitary Surveillance - ANVISA.All the treatments used presented to be within the microbiological standards recommended by ANVISA for human consumption of bean, so it is recommended bean irrigation with 100% of treated domestic effluents concentration or 50% water supply plus 50% treated effluent, thus targeting treated water for other purposes, since there are no thermotolerant coliforms or *Salmonella sp*.

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INTRODUCTION

The increase in agricultural production demands new strategies, in order to boost productivity and reduce production risks. With the growth of the world population, food production based only in the rainy season is not enough. One of the considerable challenges of current agriculture is the expansion of competitiveness and product quality, related to the preservation of water resources and the environment, allowing sustainable benefits in agricultural activities. In this context, it is relevant to evaluate and adapt each of the factors that integrate the production system, inserting the efficiency and the management of irrigation water. The bean (*Phaseolus vulgaris L.*) is one of the main crops produced in Brazil and the world, its importance exceeds the economic aspect, due to its nutritional relevance, in the culinary and the culture of several countries (Silva *et al*., 2012). Brazil is the third world producer of beans with 1.9 million tons, surpassed by India (6.2 million) and Myanmar (4.7 million). In 2018, bean yield in Brazil was 10 ton ha^{-1}, while in other countries it was 58 ton ha^{-1} (FAO, 2019). Beans are one of the main subsistence crops in the Northeast region of Brazil and one of the main factors for the productive success of the bean in this region is the ideal water supply, supplying its necessity according to the phenological stages of the crop(Kobayashi *et al*., 2016), especially in protected environment where irrigation is the main way to provide water for plant (Guimarães *et al*., 2019a; 2019b). Water scarcity in arid and semi-arid regions, as in Northeast region of Brazil, has become more and more frequent and an alternative in agriculture is the reuse of water in irrigated cultivation areas, however there is a concern with crop contamination (Zhang *et al*., 2018). Several researchers have developed works to analyze the effects of using wastewater in the production of irrigated crops, such as lettuce (Fonteles *et al*., 2015; Urbano *et al*., 2017; Gomes Filho *et al*., 2020), okra (Santos *et al*., 2018), cowpea beans (Feitosa *et al*., 2015) and chilli pepper (Silva *et al*., 2019). Based on the previous report, the objective of this study was to evaluate the microbiological quality of *BRS-Pontal* bean submitted to different irrigation depths with treated domestic effluent.

MATERIALS AND METHODS

The experiment was carried out in a protected environment of the Department of Agronomic Engineering (DAE) at the Federal University of *Sergipe* in *São Cristovão*, *Sergipe*State, Brazil (Figure 1).

Figure 1. Protected Environment where the experiment was carried out with bean irrigated with treated domestic effluent

Table 1. Values of the chemical characteristics of the water from the Sergipe Sanitation Company and the treated domestic efluente

Characteristics	Water	Treateddomesticeffluent
Total Phosphorus (mg L^{-1})	<0.037	1.81
Carbonates (mg L^{-1})	<5.22	<5.22
Bicarbonates (mg L^{-1})	115.00	175.30
pH	7.24	7.46
ElectricalConductivity (µS cm^{-1})	368.40	578.00
Total Dissolved Solids (mg L^{-1})	206.30	374.6
Nitrate (mg L^{-1})	9.80	2.23
Nitrite (mg L^{-1})	<0.0009	0.2600
AmmoniacalNitrogen (mg L^{-1})	0.12	3.72
Potassium (mg L^{-1})	1.82	8.80
Calcium (mg L^{-1})	57.09	49.01
Sodium (mg L^{-1})	30.10	42.40
SAR	5.20	7.90
Magnesium (mg L^{-1})	8.97	8.99
Sulfates (mg L^{-1})	64.00	85.00
Chloredis (mg L^{-1})	43.63	92.11

Water from the Sergipe Sanitation Company and treated domestic effluent from the Effluent Treatment Station of the Federal University of Sergipe were applied daily. The chemical characteristics of the water from the Sergipe Sanitation Company and the treated domestic effluent can be seen in Table 1.The experimental design was randomized blocks in a 3 x 4 factorial scheme with four replications. The irrigation of the bean was carried out in three different concentrations of treated domestic effluent: C1 (0% treated domestic effluent), C2 (50% domestic effluent + 50% Sergipe Sanitation Company water supply), C3 (100% treated domestic effluent) and four irrigation depths corresponding to 50% 75% 100% and 125% of crop evapotranspiration with four replications. Seeding was carried out using *BRS-Pontal* bean seeds. The used soil came from the Rural Campus at the Federal University of *Sergipe*, classified as Ultisol, which is characterized as deep to shallow; moderately well drained; very variable texture, but with a predominance of average surface texture, and clayey, subsurface texture; and has low to medium total porosity (Embrapa, 2013). The beans were grown in pots and irrigated daily. At the seven days after the germination, the thinning of the seedlings was carried out, when they presented the first pair of definitive leaves, leaving the one more vigorous. The methodology proposed by document FAO 56 was used for the estimation of the water demand of the bean. The climatic variables (temperature, relative humidity, radiation and wind speed) were monitored daily by the Automatic Weather Station located within the protected environment to support the estimation of reference evapotranspiration by the Penman-Monteith method.

The crop coefficient was established as a function of the phenological phase of the crop. After harvesting, 25 g of each sample were weighed. Samples were aseptically transferred into flasks containing 225 mL of peptone water and then decimal dilutions were made up to 10^{-6} in 0.1% peptone water. Duplicates of each sample were made. For the analysis of *thermotolerant coliforms*, aliquots of 1 mL of each dilution were inoculated in series of three tubes containing 9 mL of lactosate broth. Subsequently, the tubes were incubated at 95 °F for 48 hours and the tubes with positive reading (turbidity and gas formation) followed for the confirmatory tests. Regarding the enumeration of mesophiles, from each selected dilution, 1 mL was seeded in Petri dishes on which Plate Count Agar - PCA was added. After complete homogenization and solidification of the medium, the plates were incubated at 98 °F for 48 hours. Plates containing between 25 and 250 colonies were counted and the results were expressed in colony forming units per gram of food (*CFU* g^{-1}).In the pre-enrichment stage for the analysis of the *Salmonella*, 25 g of sample were diluted in 225 mL of lactose broth which was subsequently incubated at 98 °F for 24 hours. For the selective enrichment, after the pre-enrichment incubation, aliquots of 0.1 mL and 1.0 mL of the lactosate broth were removed, which were added in two different selective media, Rappaport-Vassiliadis and the Tetrathionate broth at 107 °F and 98 °F, respectively. This inoculum was then seeded in differential selective medium of Hektoen Enteric Agar (HE-Oxoid) and Xylose-Lysine Deoxycholate (XLD) and incubated at 98 °F for 24 hours to obtain typical colonies, that they were confirmed by biochemical tests. The methodologies for microbiological analysis of *BRS-Pontal* bean samples were performed according to those described in the *Compendium of methods for the microbiological examination offoods* (Downes and Ito, 2001).

The microbiological quality of the samples was evaluated for the population of thermotolerant coliforms (*MPN* g^{-1}), mesophiles (*CFU* g^{-1}), molds and yeasts (*CFU* g^{-1}), as well as Salmonella sp. Analyzes were carried out at the Biotechnology Laboratory of the Food Technology Department of the Federal University of *Sergipe, Brazil*. The results of the samples were presented by means of the minimum and maximum values observed for each group of microorganisms studied in their corresponding samples. The beans samples were subjected to the analysis according to the parameters recommended by Resolution 12 of January 2, 2001 by National Agency of Sanitary Surveillance - ANVISA (ANVISA, 2001).

RESULTS AND DISCUSSION

The results for Salmonella sp., Molds and yeasts, thermotolerant coliforms and mesophiles are shown in Table 2. Red Latosol and the yield and quality of lettuce after cultivation using treated wastewater for irrigation, observed the absence of coliforms and Salmonella, considering the quality treated wastewater suitable for lettuce drip irrigation. Gatta *et al*. (2015) observed that tomatoes when irrigated with wastewater from agribusiness did not suffer influence on the microbiological quality, agreeing with this work. Beneduce*et al*. (2017) suggested that the reuse of wastewater from the food industry for irrigation of agricultural crops can be applied without significantly increasing the potential health risk related to microbial quality, agreeing with this work. According to Batista *et al*. (2017), the papaya fruits produced did not show contamination by total coliforms in any of the treatments, as well as the absence of Salmonella sp, presenting no risk for fresh consumption. Silva *et al*. (2016) found that coliform counts were higher than those allowed by Brazilian legislation, when irrigating lettuce with water from reservoirs in the rural area of Caruaru city.

Table 2. Microbiological quality of BRS-Pontal bean irrigated with different proportions of treated domestic effluent and different irrigation depths

TREATMENTS	*Salmonella* sp. (25 g)	*ThermotolerantColiform* (*NMP* g^{-1})	*Mesophiles*(*CFU* g^{-1})	*Molds and yeasts* (*CFU* g^{-1})
C1L1	ABSENCE	< 3	1.2×10^2	< 10
C2L1	ABSENCE	< 3	1.8×10^2	< 10
C3L1	ABSENCE	< 3	2.5×10^2	< 10
C1L2	ABSENCE	< 3	6.5×10^2	$< 10^2$
C2L2	ABSENCE	< 3	1.2×10^4	2.0×10^3
C3L2	ABSENCE	< 3	7.3×10^6	3.0×10^2
C1L3	ABSENCE	< 3	6.3×10^4	3.1×10^3
C2L3	ABSENCE	< 3	7.5×10^3	6.0×10^4
C3L3	ABSENCE	< 3	2.3×10^3	7.2×10^3
C1L4	ABSENCE	< 3	3.2×10^4	5.6×10^5
C2L4	ABSENCE	< 3	5.9×10^3	13.3×10^3
C3L4	ABSENCE	< 3	3.6×10^3	6.1×10^4

(C1 = 0% of treated domestic efluent), (C2 = 50% of water supply + 50% of treated domestic efluent), (C3 = 100% of treated domestic efluent), (L1 = 50% of ETc replacement), (L2 = 75% of ETc replacement), (L3 = 100% of ETc replacement), (L4= 125% of ETc replacement), ETc = crop evapotranspiration.

Although there is no resolution setting maximum limits for thermotolerant coliforms and Salmonella for bean, the legume resolution was used as a comparison. The resolution determines as maximum count of *Thermotolerant coliforms* for walnuts, almonds, peanuts and the like, raw, whole or shelled stable at room temperature the value of 45 *NMPg*$^{-1}$and absence for *Salmonella sp.* According to what was previously described and observed in Table 2, the results found were lower than 3.0 *MPN* g^{-1}, taking into account the parameters in ANVISA's resolution. All the treatments used were within the standards recommended by the ANVISA's resolution, so it is recommended that the bean culture can be irrigated with the treatments that have concentration (C3), composed entirely of treated domestic effluents or 50 % water supply, plus 50% treated effluent (C2), thus targeting treated water for other purposes, since there are no *thermotolerant coliforms* or *Salmonella sp*.

It is noteworthy that the bean grains have a protective layer, the pod, which makes them less susceptible to contamination during irrigation, further favoring irrigation with treated domestic effluents. Gomes Filho *et al* (2020) observed that treated domestic wastewater may be considered a possible source of water for lettuce when it is applied to the soil in the vicinity of the root system of the crop, when they evaluated the microbiological quality of lettuce irrigated with different concentrations treated domestic effluents and irrigation depths. It was verified that the application of water in the vicinity of the root system of the plants did not affect the microbiological quality of the beans. Urbano *et al*. (2017) evaluating the physical, chemical and microbiological changes of a Dusky These results were worse than those found in this work. In relation to the population of mesophilic microorganisms, the bean samples irrigated with 100% treated domestic effluent (C3) had a higher count when compared to the other treatments, however this fact was only observed for the L2 irrigation depth (75% of the ETc) and not for the L3 (100% ETc) or L4 (125% ETc) irrigation depths (Table 2). According to Nguyen-The and Carlin (1994), the count of mesophilic microorganisms present in plants can be as high as 109 *CFUg*$^{-1}$, but it is usually 104 - 106 *CFUg*$^{-1}$. This variability occurs as a function of the climatic conditions, the structure of the crop (the presence or absence of bark), the presence of animals and insects, the injury in the plant itself or the type of irrigation. The mold and yeast population ranged from 103 to 105 *CFUg*$^{-1}$, regardless of the effluent concentration used in the treatments (Table 2). This amount, according to Pires (2014), is considered normal for this crop, since the population can vary up to 109 *CFUg*$^{-1}$.Carvalho *et al*. (2013) found values within the limit allowed by ANVISA for molds and yeasts when studying the microbiological quality of the seed meal of the sunflower. It is worth mentioning that these analyzes were performed with raw beans and that the legume passes through the cooking process to be consumed, reaching 212 to 248 ºF before consumption, which will make it safe for consumption, since, according to Oliveira *et al*. (2008) the heat treatment considerably reduces microbiological contamination.

Conclusions

All the treatments used presented to be within the microbiological standards recommended for human consumption of bean, so it is recommended bean irrigation with 100% of treated domestic effluents concentration or 50% water supply plus 50% treated effluent, thus targeting treated water for other purposes, since there are no *thermotolerant coliforms* or *Salmonella sp*. The treated domestic effluents may be used in the irrigation of the bean culture, verifying the efficiency of the domestic effluent treatment. It is necessary to take into account the choice of the appropriate management of the irrigation system, applying the effluent in the vicinity of the crop root system, avoiding the contact of the effluent directly with the plant, restriction of the type of crop to be irrigated and care at harvest, transportation and handling.

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