

## **Soil chemical parameters and microbiological quality of biquinho pepper irrigated with treated wastewater in protected environment**

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**Abstract**

*The aim of this work was to evaluate the soil chemical parameters and microbiological quality of biquinho pepper fruits irrigated with treated wastewater and different irrigation depths. The experiment was carried out in protected environment with the application of four irrigation depths corresponding to 50, 75, 100 and 125% of the crop evapotranspiration with three concentrations of 0, 50 and 100% of treated wastewater in the cultivation of biquinho pepper in pots. The experimental design was in randomized blocks in a 3 x 4 factorial scheme, totaling 12 treatments, with three replications. The soil chemical parameters were determined before and after the growing the pepper with treated wastewater. Reference evapotranspiration (ET<sub>o</sub>) was estimated by the FAO Penman-Monteith method using data obtained from an Automatic Weather Station installed inside the protected environment. The microbiological quality was analyzed by quantifying fecal coliforms and the presence of Salmonella sp. recommended by Brazilian National Agency for Sanitary Surveillance (ANVISA). All pepper samples were in accordance with the limits recommended by the legislation and, therefore, the use of wastewater in pepper irrigation did not represent a risk to public health. The soil chemical parameters were changed in different ways after the application of the treated wastewater, being motivated by both soil and wastewater chemical characteristics.*

**Keywords:** *Salmonella*; fecal coliforms; evapotranspiration; ultisol; organic matter.

**1. Introduction**

In countries and regions with water scarcity, the reuse of wastewater is an alternative for replacing fresh water resources with wastewater for purposes that do not require drinking water quality (Helmecke, Fries,

and Schulte 2020).

The use of wastewater has become an interesting option as it reduces contamination by direct discharge of wastewater into water bodies, allows a more rational use of water resources and is an alternative source to make water available for agricultural crops (Cuba et al. 2015).

The need for reuse of wastewater for irrigation has become of paramount importance for countries with water scarcity (Tripathi et al. 2016). With the increase demand for irrigation water, the use of low quality water is an alternative to human coexistence in the semi-arid, reducing the demand for available water resources (Lucena et al. 2018).

Wastewater is used for crop irrigation as an alternative to better quality water. It has now become a common practice in many countries around the world to use wastewater for irrigation. Irrigation of crops with wastewater represents opportunities and challenges with regard to their uses and the environment, mitigating water deficit crises mainly in the semi-arid regions of the world (Khalid et al. 2018).

Several researchers (Aziz 2015; Demir and Sahin 2017; Disciglio et al. 2015; Gupta et al. 2019; Khalid et al. 2018; Khaliq et al. 2017; Libutti et al. 2018; Santos et al. 2018; Urbano et al. 2017; Yassin et al. 2017; Zeeshan and Shehzadi 2019) have carried out work with the use of wastewater in the irrigation of agricultural crops and the effect on soil chemical parameters.

With the occurrence of prolonged droughts in the Brazilian semiarid region, water scarcity has influenced agriculture in this region, so that the reuse of wastewater has become an alternative for pepper irrigation (Silva et al. 2019).

There is no record by agricultural production control agencies of an accurate estimate of pepper production in Brazil because most of the peppers are grown by small farmers from several Brazilian regions. There is a wide commercial acceptance of fresh or processed pepper fruits, covering also the marketing of ornamental plants and the manufacture of cosmetics and medicines (Ferraz et al. 2016).

The biquinho pepper is one of the most used varieties by small producers seeking high productivity, acceptance by the consumer market and good financial return, being attractive mainly for its mild taste and absence of pungency (Alves et al. 2016; Heinrich et al. 2015).

According to the above, this work aimed to evaluate the soil chemical parameters and the microbiological quality of the biquinho pepper fruits irrigated with treated wastewater and different irrigation depths.

## **2. Material and Methods**

The experiment was conducted in pots growing biquinho pepper (*Capsicum chinense*), belonging to the Solanaceae group, on a metal bench in protected environment. The treatments consisted of four irrigation depths corresponding to 75, 100, 125 and 150% crop evapotranspiration (ET<sub>c</sub>) with three concentrations of treated wastewater prepared with 0% treated wastewater, 50% supply system water plus 50% treated wastewater, and 100% treated wastewater. The experimental design was in randomized blocks, in the 3 x 4 factor scheme, with four repetitions, totaling 12 treatments.

Reference evapotranspiration (ET<sub>o</sub>) was estimated using the FAO 56 Penman-Monteith standard method using data obtained from an Automatic Weather Station installed inside the protected environment. The crop evapotranspiration obtained as a function of ET<sub>o</sub> and the crop coefficient values of the pepper grown

in protected environment of 0.74, 1.39, 1.17 and 1.02 (Santos et al. 2016).

The water supply was collected from a tap that was inside the protected environment and the treated wastewater was collected weekly from a Sewage Treatment Station, consisting of a pre-treatment unit with a grid and sand box and three treatment tanks, being anaerobic, aerobic and chemical. The collection was carried out in the aerobic tank, as the former contained a very high organic load that could cause damage to the crop and the latter tank contained chemicals.

The chemical parameters of the water supply system and treated wastewater can be seen in Table 1.

Table 1. Values of the chemical parameters of the water supply system and the treated wastewater

Chemical parameters	Water supply system	Treated wastewater
Total Phosphorus (mg L <sup>-1</sup> )	<0.037	1.81
Carbonates (mg L <sup>-1</sup> )	<5.22	<5.22
Bicarbonates (mg L <sup>-1</sup> )	115.00	175.30
pH	7.24	7.46
Electrical Conductivity (μS cm <sup>-1</sup> )	368.40	578.00
Total Dissolved Solids (mg L <sup>-1</sup> )	206.30	374.60
Nitrate (mg L <sup>-1</sup> )	9.8	2.23
Nitrite (mg L <sup>-1</sup> )	<0.0009	0.26
Ammoniacal Nitrogen (mg L <sup>-1</sup> )	0.12	3.72
Potassium (mg L <sup>-1</sup> )	1.82	8.80
Calcium (mg L <sup>-1</sup> )	57.09	49.01
Sodium (mg L <sup>-1</sup> )	30.10	42.40
SAR	5.20	7.90
Magnesium (mg L <sup>-1</sup> )	8.97	8.99
Sulfates (mg L <sup>-1</sup> )	64.00	85.00
Chloredis (mg L <sup>-1</sup> )	43.63	92.11

The sowing was done in Styrofoam trays filled with vegetable soil, which contained in its composition cattle manure and castor cake. Then the Styrofoam tray was taken to a greenhouse where it had a nebulizer to facilitate the germination of the seeds, which occurred 10 days after sowing, performing the thinning leaving only the most vigorous. The seedlings were transplanted into pots with a capacity of 21 L, each containing 15 kg of soil, classified as Ultisol.

The soil chemical parameters before the application of the treatments, with biquinho pepper crop irrigated with water from the supply system and treated wastewater can be seen in Table 2.

Table 2. Chemical parameters of the soil before application of the treatments, with beak pepper crop irrigated with water from the supply system and treated wastewater

Treatments	Soil chemical parameters									
	pH	OM	Na	K	Ca	Mg	P	CEC	V	EC
75% of ETc and 0% of TWW	6.2	8.9	56.2	120	4.1	1.8	66.4	8.0	79.7	1.1
75% of ETc and 50% of TWW	5.7	13.0	74.2	220	13.3	1.0	59.5	7.5	70.1	1.0
75% of ETc and 100% of TWW	6.6	31.0	87.0	196	5.4	1.1	19	9.0	81.8	1.0
100% of ETc and 0% of TWW	6.3	13.0	0.32	0.42	4.5	1.3	80.1	8.2	80.1	0.8
100% of ETc and 50% of TWW	6.6	12.2	0.25	118	3.5	0.8	17	6.1	76.4	1.0
100% of ETc and 100% of TWW	6.9	13.3	0.23	146	4.2	1.1	58.4	7.3	80.5	1.4
125% of ETc and 0% of TWW	6.4	9.3	0.29	109	3.4	1.2	45.1	6.8	76.1	1.2
125% of ETc and 50% of TWW	6.7	12.1	0.48	170	5.1	0.3	36.9	7.5	83.4	1.2
125% of ETc and 100% of TWW	7.1	13.6	0.24	151	3.5	1.0	59.6	5.1	100.0	0.9
150% of ETc and 0% of TWW	5.9	7.4	0.53	264	5.4	1.8	77.7	10.5	80.5	1.0
150% of ETc and 50% of TWW	6.5	12.4	0.83	267	4.5	2.0	54.2	9.5	85.0	1.0
150% of ETc and 100% of TWW	6.9	13.2	0.24	102	3.7	1.2	38.8	6.7	81.5	1.0

TWW – treated wastewater; ETc – crop evapotranspiration; OM – organic matter; Na – sodium; K – potassium; Ca – calcium; Mg – magnesium; P – phosphorus; CEC – cation exchange capacity; V – base saturation; EC - electrical conductivity.

The planting fertilization was performed at the time of transplantation for all pots and 1 g pot<sup>-1</sup> of urea (N), 4.2 g pot<sup>-1</sup> of phosphorus (P<sub>2</sub>O<sub>5</sub>) and 1.5 g pot<sup>-1</sup> of potassium chloride (K<sub>2</sub>O) were applied. Until the stability of the crop, only drinking water was used, thus inserting the treated wastewater from the second phenological phase of the crop.

For cover fertilization 0.5 g pot<sup>-1</sup> of urea and 0.75 g pot<sup>-1</sup> of potassium chloride were applied 30 days after transplanting in the treatments that were irrigated with 100% water from the supply company.

For microbiological quality, fecal coliforms and Salmonella analyses were performed according to the method described by APHA (2001) and the microbiological standards established for fresh vegetables in Resolution RDC n° 12/01 of the Brazilian National Agency for Sanitary Surveillance (ANVISA), were adopted as the reference.

### 3. Results and Discussion

Microbiological analysis of biquinho peppers was performed to verify whether or not they were contaminated by supply water and treated wastewater (Table 3), based on the analyses recommended by RDC 12/01, which allows up to 102 MPN g<sup>-1</sup> for fecal coliforms and requires absence of Salmonella sp. It can be observed that fecal coliforms and Salmonella were not found in the analyzed peppers for all treatments, indicating that all samples were compliant with the legal patterns (Table 3).

Table 3. Microbiological quality of the beak peppers irrigated with supply water and treated wastewater

Treatment	Fecal coliforms (NMP g <sup>-1</sup> )	<i>Salmonella sp</i>
75% of ETc and 0% of treated wastewater	< 3	Absence
75% of ETc and 50% of treated wastewater	< 3	Absence
75% of ETc and 100% of treated wastewater	< 3	Absence
100% of ETc and 0% of treated wastewater	< 3	Absence
100% of ETc and 50% of treated wastewater	< 3	Absence
100% of ETc and 100% of treated wastewater	< 3	Absence
125% of ETc and 0% of treated wastewater	< 3	Absence
125% of ETc and 50% of treated wastewater	< 3	Absence
125% of ETc and 100% of treated wastewater	< 3	Absence
150% of ETc and 0% of treated wastewater	< 3	Absence
150% of ETc and 50% of treated wastewater	< 3	Absence
150% of ETc and 100% of treated wastewater	< 3	Absence

ETc – crop evapotranspiration

These results were similar to those reported by Gomes Filho et al. (2020a) and Gomes Filho et al. (2020b) when they analyzed lettuce and beans irrigated with treated wastewater. As well as the ones found by Batista et al. (2017), who evaluated the microbiological quality of papaya fruit produced with treated wastewater. Libutti et al. (2018) reported that the fecal coliforms populations in tomato and broccoli were not influenced by the types of water used for irrigation. The authors described that the drip irrigation system used, which avoided close contact between the water and the plant, may have contributed to these results. As found in this research, that also applied water near the root system of the crop, without direct contact with the leaves and fruit.

According to Alves et al. (2017), the microbiological quality of bananas irrigated with treated wastewater was similar to those irrigated with supply water and mineral fertilizers, however, the effects of irrigation using treated wastewater should be monitored to ensure that they do not compromise the quality of the final product or consumer acceptance.

Based on the values of the soil chemical parameters evaluated before (Table 2) and after (Table 4) the application of the treatments, it was observed that some parameters had positive variation (elevation of pH, EC, and V), negative variation (decrease of K and Na) and others with positive and negative variations (organic matter, Ca, Mg and CEC). The values of Aluminum were constant and equal to 0.08 cmolc dm<sup>-3</sup> before and after the cultivation irrigated with treated wastewater (Table 4).

It was found that the pH values of the soil increased in all treatments, probably due to base dispersion during cultivation and dissolution by irrigation, since the water used in irrigation had alkaline pH and high levels of bicarbonates and calcium when TWW was absent (Table 1). When the TWW is present, this should probably also be attributed to the pH of both the irrigation water and the TWW itself, whose high levels of calcium and sodium, in addition to chlorides, caused an increase in their respective values in the soil. This resulted in the increase in the values of sodium, potassium, calcium, phosphorous and magnesium contents

(Tables 2 and 4). Research conducted using agro-industrial wastewater in tomato cultivation has provided a slightly increase in soil pH (8.2) with respect to the initial value (7.9) probably due to the accumulation in the soil of basic cations due to the fertilising (Disciglio et al. 2015).

The high levels of salts in the soil, expressed by sodium, influenced the values of EC (Table 4), markedly in the treatment corresponding to 75% of the ETc, which can be attributed to the effects of this water (Table 1) on the soil, because it has high levels of salts and bases, contributing to the elevation of such parameters. The average values of EC in all treatments after the application of TWW (Table 4), it indicated moderate risk regarding soil salinization (Ayers and Westcot 1991). Abd-Elwahed (2018) observed that huge volumes of wastewater applied over the long term increased salinity, negatively affecting soil quality. Heras and Mañas (2020), studying the effect of wastewater on the chemical parameters of soil cultivated with olive groves and vineyards irrigated with treated wastewater, found high values of electrical conductivity in the soil.

As the pH values of both the water used for irrigation and the TWW are in the alkalinity range (Table 1), consequently the values of the aluminum contents (Table 4) were low, as they were displaced from the sorbent complex, occupied not only by the bases, but predominantly by salts, so much so that the electrical conductivity of the soil in all the treatments rose because of this behavior, after the addition of irrigation water raised the values, with greater differences when adding TWW (Tables 2 and 4), as well as for base saturation (V%).

It was observed that the sodium values showed higher decreases in treatments with lower doses of TWW combined with higher levels of available water in the soil (Table 4). This is explained by the higher water leaching capacity in this condition and associated with lower doses of TWW containing  $42.4 \text{ mg L}^{-1}$  of sodium (Table 1), reducing the soil content and cause larger reductions proportionally at the end of the research. Urbano et al. (2015) observed that the concentration of nutrients in the soil increased, including sodium, after the passage of domestic sewage reuse water through Oxisol, in the lettuce crop, with a tendency to salinate, but there was no damage to the physical properties of the soil. However, Medeiros et al. (2011) observed that sodium contents increased in all soil layers (0 to 1.0 m, evaluated every 0.2 m), in all different swine effluent dosages, but the highest contents were verified in the areas that received treated effluent, behavior contrary to that obtained in the present study, indicating in this case, lower propensity to soil salinization. Excess sodium in soils causes harmful physical conditions for growth and, mainly, for the development of the root system, because in low concentration sodium acts as dispersant and in high concentration as a binder, destroying the soil structure. Generally, sodium soils, which have a high sodium content, cause the reduction of nitrogen mineralization, directly affecting plant growth (Dias and Blanco 2010). Thus, crops can be directly affected when high concentration sodium in irrigation water becomes a toxic element for the plant and indirectly when it causes unfavorable physical conditions for the development of the root system (Oliveira et al. 2013).

It was also observed that for the pH range of the values obtained in the soil there was an increase in the organic matter contents in the soil, due to two conjugated factors. The first factor is the TWW as source of organic matter and its preservation in the soil due to the pH range of the soil (Table 1), the irrigation water and the TWW, corresponding to alkalinity, a situation that preserves more this organic matter, since in this condition, there is less biological activity resulting in less oxidation of these organic sources, contributing

to the elevation of the contents (Tables 2 and 4). This organic matter, due to its high specific surface, works as adsorption sites, not only of salts, but other chemical elements, decreasing their leaching to deeper layers of the soil, contributing to the maintenance of higher levels of Mg, P and Ca, resulting in higher values of CEC and V% (Tables 2 and 4).

The increase in organic matter content may have been derived from the contribution of TWW, as it has a total dissolved solids content of  $374.60 \text{ mg L}^{-1}$ , an aspect that contributes to the increase in content, which associated with higher levels of available soil water, by decreasing the proportion of air in the soil, decreasing biological activity and processes related to decomposition and oxidation of organic matter, resulting in smaller reductions, even at higher concentrations of TWW added to the soil (Table 4).

Finocchiaro and Kremer (2010) observed an inverse relationship between soil microbial activity and the deposition of treated wastewater effluent when compared to river water although they observed that the wastewater had twice the electrical conductivity and four times the sodium concentration compared to river water. In this study the average electrical conductivity obtained was around  $2.2 \text{ dS m}^{-1}$  at the end of the experiment, a condition that would stimulate soil microbial activity.

The increase of phosphorus contents in all treatments is probably due to the greater solubilization of this chemical element with the irrigation water, so much so that higher final contents of this element were observed in the soil for the largest irrigation depths, being potentialized by the addition of TWW, since this material is the source of this element. Although phosphorus has low mobility in the soil, irrigation results in greater solubility because of the greater availability of water and a high soil infiltration rate, which favored its release and coupled with low mobility in the soil profile, resulted in the end in greater concentration.

Belaqziz et al. (2016), investigating the effect of direct amendment of olive mill wastewater, observed an important increase in soil chemical parameters, namely electric conductivity (EC), phosphorus, sodium and organic matter. Yassin et al. (2017) found that the mean levels of P in the soil showed an increasing trend towards the end of growing season with the highest concentrations in the soil irrigated with treated wastewater.

#### **4. Conclusions**

All the fruits samples were in accordance with the parameters officially established by Brazilian Health Regulatory Agency, as well as that treated wastewater may potentially be used as alternative irrigation water without deleteriously affecting the microbiological safety of biquinho pepper.

The soil chemical parameters were changed in different ways after the application of the treated wastewater, being motivated by both soil and wastewater chemical characteristics.

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