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ORIGINAL RESEARCH

Treated domestic wastewater for water and nutrient supply in strawberry trough culture system

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Abstract

Purpose: This study seeks to assess the cultivation and production of strawberries (cv. San Andreas) under substrate conditions, utilizing treated wastewater as an alternative water source and supplemented with mineral fertilizers.

Method: Conducted in greenhouse conditions, the experiments comprised three treatment groups: (PWF) potable water + mineral fertilizers, (TWF) treated wastewater + mineral fertilizers, and (TW) treated wastewater only.

Results: Statistical analysis revealed that using treated wastewater alone (TW) resulted in low productivity and subpar fruit quality. In contrast, the TWF solution demonstrated robust fruit productivity and quality, comparable to the conventional cultivation system using potable water and mineral fertilizers (PWF). *Escherichia coli* analysis showed no contamination in the fruit samples, confirming the safety of this system employing substrate cultivation instead of conventional soil cultivation. Additionally, the use of treated wastewater led to a 13.1% reduction in the requirement for mineral fertilizers and conserved approximately 4,500 L of potable water for irrigation.

Conclusion: These findings underscore the viability of employing treated domestic wastewater for strawberry cultivation, ensuring safe fruit production and upholding high-quality standards.

Keywords: *Fragaria x ananassa* Duch, Water recycling, Nutrient recovery, Fertigation, Greenhouse cultivation, Fruit production.

Introduction

The concern for the environment has prompted several countries to prioritize sustainability, aiming to balance development with the proper management of natural resources. While water is considered a natural and renewable resource, it can be scarce or abundant in different regions globally (Urbano et al., 2017). According to Wainer et al. (2014), the agricultural sector is responsible for consuming 70% of the world's available freshwater. By 2050, the demand for water in this sector is projected to increase by 19%, while the volume of precipitable water may decrease by 10 to 20% due to climate change.

The lack of basic sanitation and the use of pesticides in modern agriculture have led to an unsustainable global impact on water quality (Carvalho et al., 2019). Therefore, alternatives like treated domestic wastewater and nutrient recovery are being explored. The use of treated domestic wastewater for agricultural irrigation has been widely adopted in countries such as the United States, Israel, and Japan, among others. This practice provides a consistent water source and essential nutrients for agriculture (Tabatabaei et al., 2020; Cova et al., 2021; Renai et al., 2021). Treated domestic wastewater has the potential to be a valuable source of water in agriculture, providing nutrients, organic matter, and other essential components. This approach could help reduce the demand for high-quality water used in irrigation and preserve existing water resources (Asgharnejad et al., 2021; Cova et al., 2021).

Additionally, in some essential horticultural practices, growers often prefer substrates over soil cultivation to enhance human well-being and achieve more efficient crop cultivation and production (Santos et al., 2019; Becker et al., 2020). Coconut fiber stands out as a challenging substrate due to its good physical properties, non-reactivity with fertilization nutrients, long durability without changing physical characteristics, sterilization possibilities, abundance of renewable raw materials, and low cost for producers (Carrizo et al., 2002). Combining the trough culture system with coconut fiber offers advantages in reducing fungal and disease problems, contributing to a robust and balanced root system (Karagöz et al., 2022).

The integration of soilless cultivation with treated domestic wastewater emerges as an excellent alternative for growing strawberries (Recamales et al., 2007; Figueiredo et al., 2021). Strawberries are among the most highly consumed small fruits globally, with significant added value (Machado et al., 2018; Andreatta et al., 2020). However, challenges such as climatic and soil conditions, along with the imperative to increase sustainability in production systems, hinder the expansion of cultivation areas. Current cultivation practices, relying on soil with

high inputs of water, nutrients, and pesticides, may lead to contamination of fruits and by-products with pesticides, particularly in tropical regions with higher temperatures and excess humidity (El-Sheikh et al., 2023). Recent studies have demonstrated the benefits of using diluted treated wastewater for irrigation in various crops, including strawberries, both in soil and soilless conditions. This approach ensures safe fruit production, mitigating risks associated with contaminants such as *Escherichia coli* (*E. coli*) and heavy metals (Urbano et al., 2017; Carvalho et al., 2018; Figueiredo et al., 2021; Bakari et al., 2022; Al-Karablieh et al., 2024). Emphasizing the importance of research focused on facilitating the use of treated domestic wastewater in substrate cultivation systems, this technique offers an alternative to potable water for irrigation. It serves as a source of mineral nutrients that would otherwise be discharged into water bodies, ensuring productivity, quality, and benefits for vegetable cultivation, such as strawberries (Urbano et al., 2017; Figueiredo et al., 2021).

Given the above, this study aims to evaluate the viability, productivity, and quality of the San Andréas strawberry cultivar (*Fragaria x ananassa* Duch). The cultivation is conducted in a substrate using a solution derived from treated domestic wastewater, supplemented with mineral fertilizers.

Material and methods

Experimental design

Strawberries (*Fragaria x ananassa* Duch) were cultivated in a greenhouse located at the Department of Natural Resources and Environmental Protection (DRNPA) within the Center for Agricultural Sciences (CCA) at the Federal University of São Carlos (UFSCar), Araras campus, São Paulo State, Brazil, between 2021 and 2022. The precise coordinates were 22°18' 53.23"S latitude, 47°23' 00.91"W longitude, and an altitude of 629 m. According to the Köppen-Geiger classification, the local climate is labeled as "Cfa," indicating a humid subtropical climate with well-defined dry (April to September) and rainy (October to March) seasons (Medeiros et al., 2021).

The selected strawberry cultivar for this study was San Andréas, obtained from Silvano Mudás de Hortaliças, a certified seedling supplier located in Pouso Alegre City, Minas Gerais State, Brazil. The greenhouse, measuring 20 × 6.40 × 5 m (length × width × height), featured a metallic structure with an arched roof covered in transparent polyethylene. The sides were enclosed with shade cloth, and the internal layout included 12 cultivation benches (2.0 × 1.8 m; length × width), divided into four blocks. Each bench comprised four cultivation profiles measuring 0.32 × 0.19 m (width × height), with spaces of 0.30 m, 0.25 m, and 0.70 m between cultivation profiles, plants, and benches, respectively. Coconut fiber served as the substrate in a trough culture system for cultivation.

The growing channels, constructed from polystyrene material, were stabilized on two metal L-shaped brackets. The upper surface of the growing trough was covered with plastic mulch, allowing for the insertion of strawberry plantlets through the mulch. This setup facilitated the absorption of the nutrient solution applied through fertigation into the substrate.

Sampling involved 16 plants per growing channel (2 rows per trough), totaling 64 plants per bench, 256 plants per treatment, and 768 plants overall. The experimental design was completely randomized, with three treatments and four replications (plots). Each plot was subdivided into two subplots, represented by two channels and four rows of plants, amounting to 32 plants. Within each subplot, 18 plants were in the useful area, while the remaining plants served as guard plants. Fig. 1 provides an overview of the strawberry substrate cultivation system.

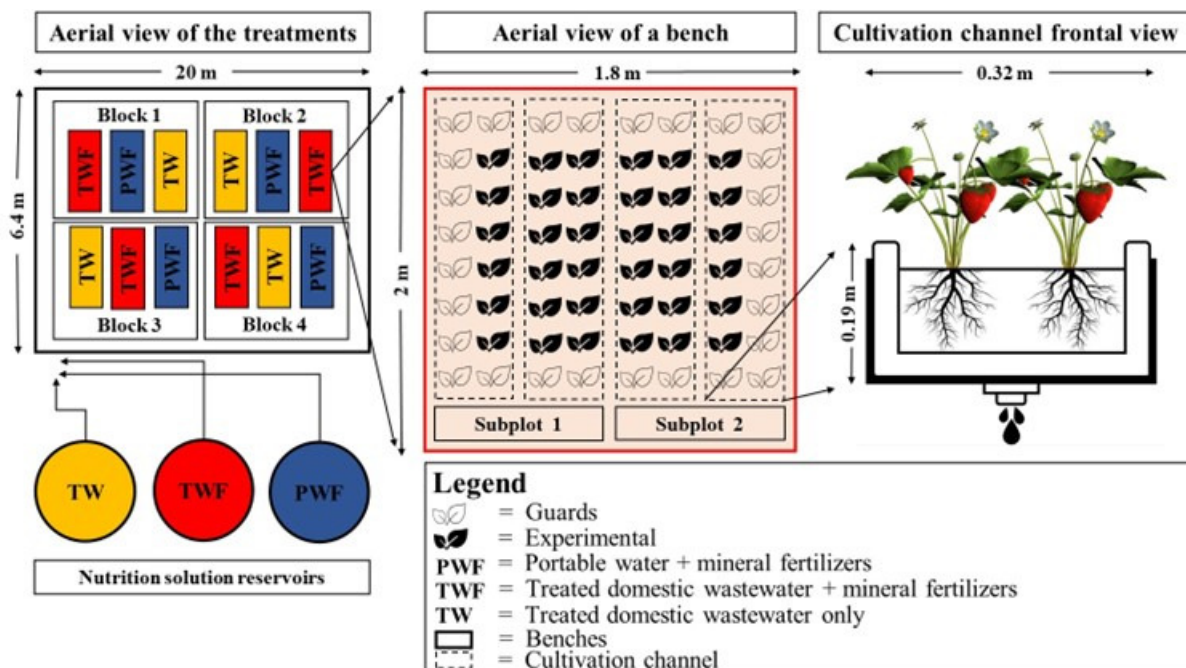


Fig. 1 Diagram of the strawberry substrate cultivation system.

The employed treatments were as follows: (PWF) - potable water + mineral fertilizers, with the mineral fertilizers quantified following the method proposed by Fernandes Junior et al. (2002) (see Table 1); (TWF) - treated domestic wastewater supplemented with mineral fertilizers, where the effluent underwent chemical analysis for mineral fertilizer supplementation, mirroring the procedure in PWF; and (TW) - treated domestic wastewater only.

Each treatment was equipped with a 500 L reservoir for storing the nutrient solution, featuring an independent pumping and irrigation system to administer different nutrient solutions. Fertigation was executed using drip irrigation, employing self-compensating emitters spaced 0.25 m apart, with a flow rate of 3.5 Lh^{-1} and a service pressure ranging from 50-350 kPa. To monitor substrate moisture and electrical conductivity (EC), two capacitance probes (Teros 12 model – METER) with 0.055 m rods were installed for each treatment. Additionally, an ATMOS 41 model - METER weather station was set up to measure temperature and relative humidity.

Table 1. Recommendations for strawberry cultivation solutions.

Nutrient	Vegetative phase (mgL ⁻¹)	Fruiting phase (mgL ⁻¹)
N (Nitrogen)	102.67	94.40
P (Phosphorus)	39.99	39.99
K (Potassium)	116.13	140.76
Ca (Calcium)	76.19	76.19
Mg (Magnesium)	27.47	27.47
S (Sulfur)	36.23	36.23
Fe (Iron)	1.79	1.79
Mn (Manganese)	0.55	0.55
B (Boron)	0.33	0.52
Zn (Zinc)	0.20	0.20
Cu (Copper)	0.08	0.08

Source: Adapted from Fernandes Junior et al. (2002).

Wastewater treatment

The treated domestic wastewater utilized in the experiment was sourced from a wastewater treatment plant (WWTP) on the Araras campus of UFSCar. To optimize waste removal and maximize nutrient utilization from the effluent, the WWTP was divided into three units (refer to Fig. 2). The wastewater treatment process involves multiple units. In the initial unit, the raw effluent is directed to a grease trap, where the majority of solids are retained. It then proceeds to a septic tank, where the remaining solids settle and undergo anaerobic digestion at the tank's bottom, facilitated by gravity.

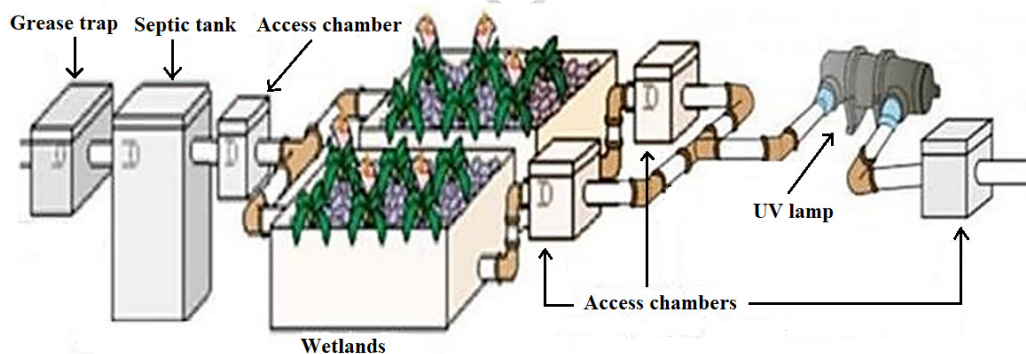


Fig. 2 Diagram of the WWTP on the Campus of Araras, UFSCar.

Source: Garay et al. (2021).

Proceeding to the second unit, the effluent is conveyed through a polyvinyl chloride (PVC) pipeline to a *wetlands* tank. This tank is filled with crushed stones and features Papyrus plants (*Cyperus alternifolius*), which thrive in moist and saturated soils and have proven to be effective in domestic wastewater treatment (Cuba et al., 2015; Souza et al., 2015). In the final unit, the effluent is transported through a pipeline and exposed to ultraviolet (UV) radiation at $0.43 \text{ mW}\cdot\text{s}^{-1}\cdot\text{cm}^{-2}$. This UV dose aids in reducing the pathogenic load. The treated effluent is then stored in an equalization tank until used in the experiment.

Measurement of quality indicators and statistical analysis

Following the treatment, the nutrient concentration in the effluent serves as the basis for managing the nutritional needs of strawberries in the TWF and TW treatments. Triplicate analyses are conducted before preparing the fertigated nutrient solution, adhering to the methods outlined in the Standard Methods for Water and Wastewater Examination (APHA, 2012). Parameters such as pH, electrical conductivity (EC), turbidity, total nitrogen (TN), total phosphorus (TP), potassium (K), calcium (Ca), magnesium (Mg), total organic carbon (TOC), sodium (Na), total coliforms (TC), and thermotolerant coliforms (*E. coli*) are scrutinized.

The sodium adsorption ratio (SAR) is calculated as the ratio of sodium (Na) divided by the square root of calcium (Ca) plus magnesium (Mg) (Lesch and Suarez, 2009) (Equation 1).

$$\text{SAR} = \frac{\text{Na}}{\sqrt{\frac{\text{Ca} + \text{Mg}}{2}}} \quad (1)$$

where:

SAR - Sodium adsorption ratio;

Na – Sodium concentration (mmolL⁻¹);

Ca – Calcium concentration (mmolL⁻¹);

Mg – Magnesium concentration (mmolL⁻¹).

To ensure optimal conditions for strawberry growth, pH levels are monitored at the nutrient solution reservoirs (inlet) and the drained solution after cultivation (outlet) in the system. Weekly pH averages are recorded for each treatment, and new nutrient solutions are prepared every 10 to 15 days based on the specific requirements of the strawberries. A total of 13 boxes, each with a capacity of 350 L, are prepared for a study period of 26 weeks.

Electrical conductivity (EC) is also monitored at the inlet, mid-point (nutrient solution in the cultivation profile substrate), and system outlet. Weekly data are collected for each treatment, and corrective measures are taken if salt concentrations accumulate in the coconut fiber. This involves adding water to the nutrient solution reservoirs to dilute the nutrient concentration applied through fertigation, resulting in the leaching of salts in the fiber. Fernandes Junior et al. (2002) state that the recommended EC range is between 1.3 and 1.5 dSm⁻¹. pH levels are the only parameters monitored.

Throughout the study, 35 weekly harvests are conducted following crop management practices. Strawberries are harvested when the fruits reach approximately 70% red or full ripeness, following the recommendation of Fernandes Junior et al. (2002). Qualitative and quantitative traits of the strawberries are evaluated using one randomly selected average-sized fruit from the useful area of each subplot, totaling eight fruits per treatment and 24 fruits in total. The fruits are weighed using an analytical balance to determine their fresh weight in grams (g). The number of fruits is counted, and the length and diameter of the fruits per plant are measured using a caliper. Total soluble solids (°Brix) are analyzed by macerating one strawberry at a time from each subplot. Two drops of the macerated juice are placed on the lens of a portable refractometer (KASVI, model K52-032), and the °Brix reading is taken by directing it against the light. The respective values are expressed in degrees. Microbiological analysis of strawberries follows the methodology described in Normative Instruction number 30 from the Ministry of Agriculture, Livestock, and Supply - MAPA (Brasil, 2003). Random average-sized strawberries are collected from the effective area of each plot, with four strawberries per treatment and 12 fruits analyzed in triplicate.

Statistical analysis for pH, EC, relative humidity, and temperature parameters is presented as means and coefficient of variation (%). Plots are created using ORIGIN 6.0 software (Origin, MICROCAL Software, Inc., version: 6.0; Northampton, Massachusetts - USA. 2000). Variables, including the number of fruits, fresh weight, diameter, length, and °Brix, are analyzed using R software (R Core Team, 2021). They are initially subjected to analysis of variance (ANOVA), and a pairwise comparison of treatment means is performed using the Tukey test. The normality assumption is verified using the Shapiro-Wilk test. The significance level for all analyses is set at 5%.

Results and discussion

Analysis of the treated domestic wastewater

The analysis of the treated domestic wastewater from the wastewater treatment plant (WWTP) at CCA/UFSCar revealed lower concentrations of mineral nutrients compared to the recommendations of Fernandes Junior et al. (2002) for strawberry substrate cultivation (see Table 2). The average results and standard deviations presented were derived from two characterizations conducted in triplicate during the experiment.

Table 2. Average results of the physical-chemical and biological characterization of treated domestic wastewater used for strawberry irrigation.

Parameter	Unit	Average value	Standard deviation
pH	-	7.64	0.02
Electrical Conductivity (EC)	dSm ⁻¹	0.38	0.01
Turbidity (TUR)	NTU	1.17	0.04
Total Nitrogen (TN)	mgL ⁻¹	19.60	0.49
Total Phosphorus (TP)	mgL ⁻¹	40.24	0.35
Potassium (K)	mgL ⁻¹	7.23	0.12
Calcium (Ca)	mgL ⁻¹	16.12	0.70
Magnesium (Mg)	mgL ⁻¹	10.67	0.58
Total Organic Carbon (TOC)	mgL ⁻¹	13.86	0.32
Sodium (Na)	mgL ⁻¹	33.27	0.49
Sodium Adsorption Ratio (SAR)	-	1.14	0.03
Total Coliforms (TC)	MPN 100 mL ⁻¹	616.7	*
Thermotolerant Coliforms (<i>E. coli</i>)	MPN 100 mL ⁻¹	19.4	*

* No standard deviations were provided for these averages.

According to Figueiredo et al. (2021), the concentrations of mineral nutrients in treated domestic wastewater can be influenced by the effluent's origin and the treatment steps it undergoes. The study conducted at the WWTP of CCA/UFSCar found that TN, TP, and K concentrations were low at the inlet. Although the wetlands tank reduced mineral nutrients by over 70%, it could not remove Ca and Mg (Oliveira et al., 2019). Consequently, the mineral nutrient levels in the WWTP wastewater were insufficient for strawberry cultivation.

The mineral nutrients Ca, Mg, and Na are directly related to the sodium adsorption ratio (SAR). Based on the established concepts by Cordeiro (2001), the SAR value (1.14 ± 0.03) is below 1.5. Thus, the treated domestic wastewater poses no risks to the strawberry substrate cultivation system. Antunes et al. (2016) emphasized that

pH values above 7 in nutrient solution preparations can lead to the formation of precipitates such as calcium carbonate, magnesium carbonate, and some low-solubility mineral phosphates when applied through fertigation. The pH value obtained for the domestic wastewater averaged 7.64 ± 0.02 . When diluted with mineral fertilizers in nutrient solution preparation, no precipitation of low-solubility nutrients occurred, indicating its suitability for the adopted system.

Gonçalves et al. (2016) suggested that the water used in nutrient solutions should have an electrical conductivity (EC) below 0.4 dSm^{-1} , considering the high sensitivity of strawberry crops. The domestic wastewater had an average EC of $0.38 \text{ dSm}^{-1} \pm 0.01$, falling within the recommended range for strawberry cultivation.

Microbiological characterization included the analysis of total coliforms (TC) ($616.70 \text{ MPN } 100 \text{ mL}^{-1}$) and *E. coli* ($19.40 \text{ MPN } 100 \text{ mL}^{-1}$) to determine water quality. According to Brasil (2005), *E. coli* is an indicator of contamination, exclusive to the human intestinal tract or homeothermic animals. The obtained values for TC and *E. coli* indicate a low microbial load, attributed to the third stage of the WWTP treatment system. Oliveira et al. (2019) stated that ultraviolet light has a removal capacity of 99.98% for TC and 99.99% for *E. coli*.

The joint resolution issued by the Secretariats of Health and Infrastructure and Environment of São Paulo State stipulates that treated wastewater should have a turbidity of $\leq 2 \text{ NTU}$ (Brasil, 2020). The obtained turbidity value ($1.17 \text{ NTU} \pm 0.04$) meets this standard. However, despite meeting the domestic wastewater quality standard, it cannot be used for cultivating leafy or fruit vegetables in Brazil (Figueiredo et al., 2021).

Nutrient solutions in strawberry substrate cultivation system

Monitoring pH and electrical conductivity (EC) in substrate cultivation systems is essential as the drained nutrient solutions are not reused. Changes in pH and EC can occur during crop growth and development (Gonçalves et al., 2016). The pH monitoring at the inlet and outlet of the nutrient solution revealed fluctuating values throughout the cultivation of strawberries. As stated by Sausen et al. (2020), this variability is expected in trough culture systems, as nutrient solutions lack buffering capacity, causing pH to vary over time and not remain constant within the recommended range.

At the system inlet (Fig. 3A), the average pH values were $6.5 (\pm 4.6)$, $6.5 (\pm 2.6)$, and $7.6 (\pm 3.6)$ for the TWF, PWF, and TW treatments, respectively. In contrast, the TWF treatment exhibited a pH of 6.9 at the system outlet with a coefficient of variation of ± 3.5 . The PWF treatment, on the other hand, had a pH of $7.1 (\pm 3.6)$, while the TW treatment, which involved only treated domestic wastewater, had an average pH of $7.4 (\pm 4.0)$ (Fig. 3B).

In summary, at the system inlet, only the TWF (6.5) and PWF (6.5) treatments remained within the recommended range, while the TW treatment had a pH above the recommended range (7.6). At the outlet, the average pH values for all treatments were above the recommended range — TW (7.4), TWF (6.9), and PWF (7.1).

At the system inlet (Fig. 4A), the average EC values were $1.53 \text{ dSm}^{-1} (\pm 5.7)$, $1.52 \text{ dSm}^{-1} (\pm 6.7)$, and $0.39 \text{ dSm}^{-1} (\pm 19.7)$ for TWF, PWF, and TW, respectively. Monitoring the EC of the nutrient solution remaining in the cultivation profiles showed values of $1.23 \text{ dSm}^{-1} (\pm 20.9)$ for PWF, $1.22 \text{ dSm}^{-1} (\pm 21.5)$ for TWF, and $0.25 \text{ dSm}^{-1} (\pm 18.2)$ for TW (Fig. 4B). Finally, at the system outlet, the average EC values were $1.29 \text{ dSm}^{-1} (\pm 23.2)$ for TWF, $1.25 \text{ dSm}^{-1} (\pm 29.9)$ for PWF, and $0.25 \text{ dSm}^{-1} (\pm 11.5)$ for TW (Fig. 4C).

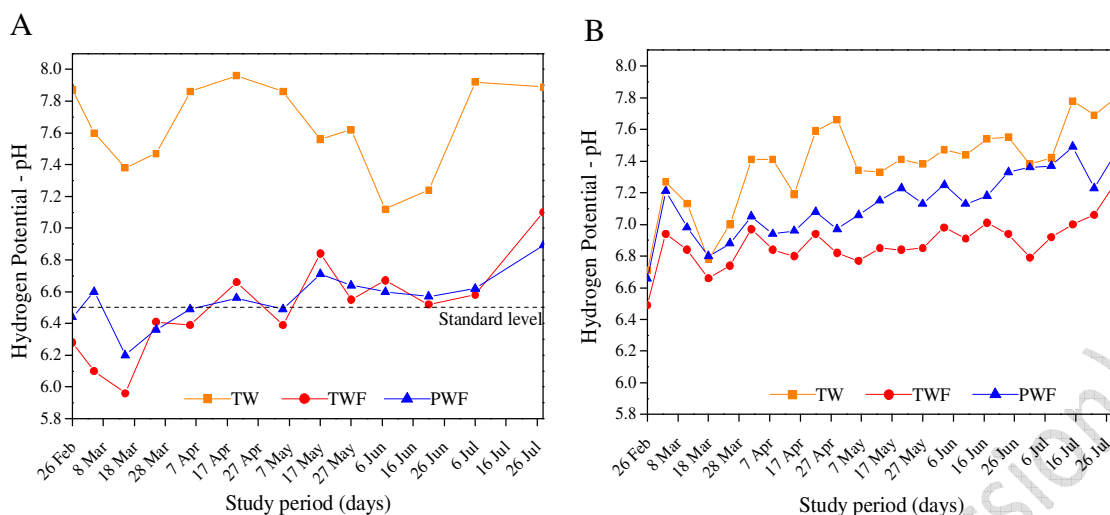


Fig. 3 Monitoring of average pH in nutrition solutions for strawberry substrate cultivation: (A) pH values in the nutrient solution input over days, being the reference standard level was considered according to the criteria established by Antunes et al. (2016). (B) pH values in the nutrient solution outlet over days.

PWF: Potable water plus mineral fertilizers; TWF: Treated domestic wastewater supplemented with mineral fertilizers; TW: Treated domestic wastewater only.

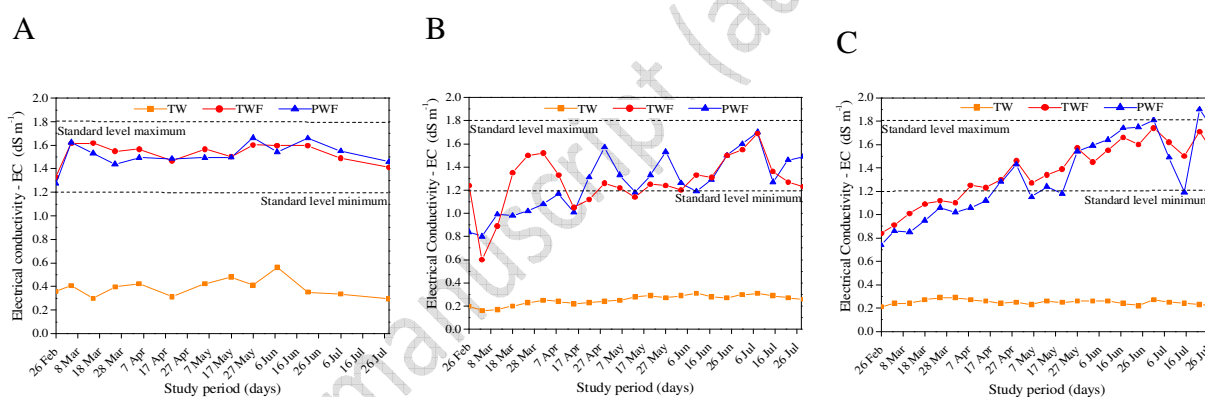


Fig. 4 Monitoring of average electrical conductivity (EC) in nutrition solutions of strawberry substrate cultivation: (A) EC values in the nutrient solution input over days; (B) EC values in the nutrient solution cultivation channel; (C) EC values in the nutrient solution outlet over days.

PWF: Potable water plus mineral fertilizers; TWF: Treated domestic wastewater supplemented with mineral fertilizers; TW: Treated domestic wastewater only.

The EC values observed in PWF and TWF were within the range recommended by Gonçalves et al. (2016). These authors suggested that the EC of the nutrient solution at the inlet should be between 1.2 and 1.8 dS m^{-1} to avoid adverse effects on plant growth. They also emphasized that fluctuations in EC, when comparing PWF and TWF to TW, are related to the absorption of mineral nutrients by the crops, leading to changes in the nutrient solution and consequently influencing EC levels.

The consistently low EC values measured in TW throughout all stages indicate that the treated domestic wastewater lacks the necessary mineral nutrients for strawberry cultivation. This nutrient deficiency is visibly evident in the low plant yield (Fig. 4). Therefore, combining a low EC below 0.4 dS m^{-1} and a pH above the

recommended range in TW directly impacts strawberry growth, development, and yield (Antunes et al., 2016; Gheyi et al., 2016).

Water and mineral fertilizer savings

Analyzing treated domestic wastewater from the wastewater treatment plant (WWTP) at CCA/UFSCar provided valuable information on the mineral nutrient content. This data was utilized to calculate the water and mineral fertilizers needed for the TWF and PWF treatments. A total of 13 nutrient solution tanks, each with a capacity of 350 L, were prepared for strawberry cultivation in each treatment (Table 3).

Table 3. Amounts of mineral fertilizers applied to PWF and TWF treatments and respective savings.

Mineral fertilizer	Applied amount		Savings (%)
	PWF (kg)	TWF (kg)	
Ca (NO ₃) ₂ (Calcium nitrate)	2.502	1.775	29.1
KNO ₃ (Potassium nitrate)	0.910	1.047	15*
KH ₂ PO ₄ (MKP - Monopotassium phosphate)	0.910	0.546	40
NH ₄ H ₂ PO ₄ (MAP - Monoammonium phosphate)	--	0.205	100*
K ₂ SO ₄ (Potassium sulfate)	0.683	1.001	46.6*
MgSO ₄ (Magnesium sulfate)	1.593	1.160	27.2
Total	6.598	5.733	13.1

PWF: Potable water plus mineral fertilizers; TWF: Treated domestic wastewater supplemented with mineral fertilizers; *: Increase in mineral fertilizer consumption; --: Without nutrient applications.

By adhering to the recommendations of Fernandes Junior et al. (2002) for strawberry cultivation, it was observed that the use of treated domestic wastewater, combined with mineral fertilizers, resulted in a 13.1% reduction in the overall usage of mineral fertilizers in the cultivation system. Specifically, the application of treated domestic wastewater supplemented with mineral fertilizers (TWF) led to significant savings of 29%, 27%, 14%, and 14% in the consumption of macronutrients Ca, Mg, N, and P, respectively. However, there was a slight increase in the demand for potassium (K) (3%) and sulfur (S) (2%), aligning with the recommended nutrient requirements for strawberry cultivation (Fernandes Junior et al., 2002) (Table 4).

Table 4. Savings in mineral fertilizers applied in PWF and TWF treatments.

Element	Applied amount		Savings (%)
	PWF (kg)	TWF (kg)	
N (Nitrogen)	0.50	0.43	14
P (Phosphorus)	0.47	0.41	14
K (Potassium)	1.22	1.26	3*
Ca (Calcium)	0.48	0.34	29
Mg (Magnesium)	0.14	0.10	27
S (Sulfur)	0.31	0.32	2*
Total	3.13	2.85	9

PWF: Potable water plus mineral fertilizers; TWF: Treated domestic wastewater supplemented with mineral fertilizers; *: Increase in mineral fertilizer consumption; --: Without nutrient applications.

When considering water conservation, the analysis of the 13 nutrient solution preparations (350 L each) for TWF and TW treatments revealed significant water savings during strawberry cultivation. Using treated domestic wastewater for agricultural purposes resulted in a total water saving of approximately 9,100 L of potable water. This calculation considers the application of 4,550 L of treated domestic wastewater for each treatment (TWF and TW). Consequently, the effluent can be effectively utilized instead of being directly discharged into water bodies, preserving potable water resources and reducing environmental impact. Figueiredo et al. (2021) demonstrated that, in addition to the savings in mineral fertilizers, the use of treated domestic wastewater provided substantial benefits to the crop by conserving valuable water resources.

Quantitative analysis of strawberry fruit

Environmental factors, such as temperature and relative humidity, directly impact strawberry cultivation. The average temperature recorded during the study period was 21.3 °C with a coefficient of variation of ± 8.7 °C. Similarly, the average relative humidity was 57% with a variation of $\pm 20\%$ (Fig. 5).

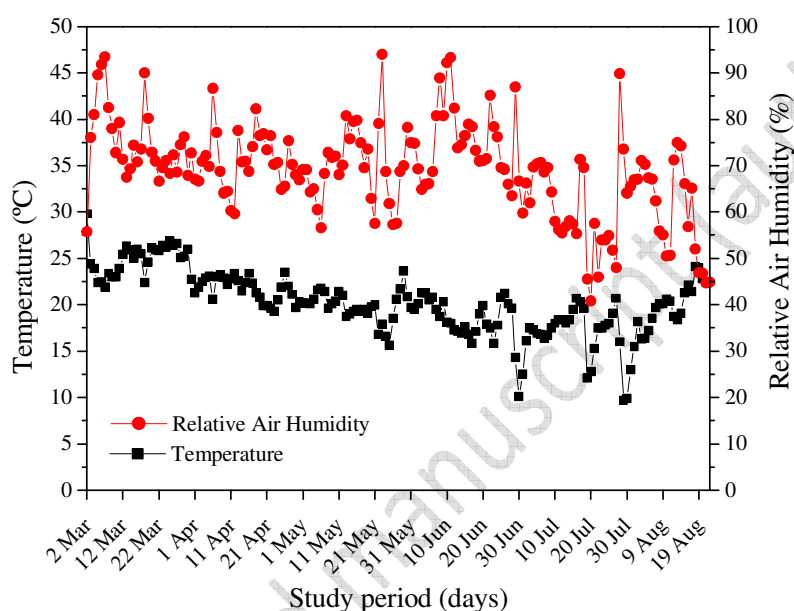


Fig. 5 Changes in temperature and relative humidity in strawberry cultivation.

According to Lopes et al. (2019), the optimal temperature range for strawberry cultivation is between 15 and 28 °C. Deviations from this range can negatively affect plant yield. Regarding relative humidity, strawberries thrive at around 60%, as higher levels can contribute to fruit and leaf diseases, besides reducing sugar content ($^{\circ}$ BRIX) in fruits. Although there was some variation in this study, the temperature (21.3 °C) and relative humidity (57%) remained within the recommended range for successful strawberry cultivation.

The ANOVA analysis showed a significant difference in the number and fresh weight (kg) of fruits when comparing PWF, TWF, and TW treatments in strawberry cultivation. Further comparisons using the Tukey mean test revealed the differences between the treatments and allowed us to identify which treatment(s) resulted in the best production/productivity of strawberry fruits. The results of the Tukey test are presented in Table 5.

Table 1. Summary of analysis of variance values, Tukey test, and Shapiro-Wilk test for strawberry cultivation methods referring to the number of fruits and fresh mass.

Treatment	Number of fruit/subplot – NF	Fresh mass (kg/subplot)
PWF	562 a	6.131 a
TWF	538 a	5.917 a
TW	97 b	0.749 b
----- ANOVA -----		
p-value	5.12 e ⁻¹⁵ *	4.45 e ⁻¹³ *
CV (%)	12.24	16.66
----- Shapiro-Wilk -----		
p-value	0.90	0.95

TWF: Treated domestic wastewater plus mineral fertilizers; PWF: Potable water supplemented with mineral fertilizers; TW: Treated domestic wastewater; *: Significant (p-value<0.05); CV: Coefficient of variation (%).

The mean comparisons revealed that PWF had an average of 562 fruits per subplot and 31 fruits per plant, which did not significantly differ from TWF, with an average of 538 fruits per subplot and 30 fruits per plant. However, TW performed significantly less than the other treatments, averaging 97 fruits per subplot and 5 fruits per plant. The Shapiro-Wilk test indicated that the residuals followed a normal distribution, and the coefficient of variation between subplots was ± 12.24 .

Regarding fresh weight, PWF had an average of 6.131 kg per subplot and 0.341 kg per plant, which was not significantly different from TWF, with an average of 5.917 kg per subplot and 0.329 kg per plant. In contrast, TW had unsatisfactory results, with an average of 0.749 kg per subplot and 0.042 kg per plant. The Shapiro-Wilk test confirmed that the residuals followed a normal distribution for each subplot, and the coefficient of variation was ± 16.66 .

Richter et al. (2018) studied strawberry production and fresh weight using different cultivation systems. They found that the San Andrés cultivar had the most significant potential output in the number of fruits and mass (kg) per plant, when compared to the level of production in the soil and with other cultivars such as Albion and Captola, which present good results in soilless cultivation systems. In a similar study, Piovesan and Hojo (2020) also assessed the performance of the San Andrés cultivar using different substrates, and cultivation in coconut fiber substrate showed the highest production potential. Figueiredo et al. (2021) investigated strawberry production in a hydroponic system using the same treatments (TW, TWF, and PWF). They found that the fresh weight of strawberries in the PWF (89.55 g) and TWF (81.82 g) treatments outperformed the TW treatment (23.76 g). Our cultivation method and proper nutrient solution management allowed us to achieve higher fresh weight and fruit numbers than their findings.

Limited availability of mineral fertilizers combined with a high pH (TW treatment) can negatively affect the cultivar's performance and limit strawberry production potential. However, when treated domestic wastewater is supplemented with mineral fertilizers (TWF treatment) and maintained at an optimal pH level for the cultivar, the production level becomes comparable to that of the conventional treatment (PWF treatment).

The ANOVA results for fruit diameter and length (cm) throughout the cultivation period indicated a significant difference between the PWF and TWF treatments compared to TW. To determine which treatments differed, the Tukey test was applied to compare the means and identify the treatments that yielded the best results (Table 6).

Table 6. Summary of analysis of variance values, Tukey test, and Shapiro-Wilk test for strawberry cultivation methods referring to fruit length and diameter.

Treatment	Length (cm)	Diameter (cm)
TWF	3.9 a	2.85 a
PWF	3.8 a	2.82 a
TW	2.9 b	2.29 b
----- ANOVA -----		
p-value	2.87 e ⁻¹⁰ *	1.65 e ⁻¹⁰ *
CV (%)	4.59	3.81
----- Shapiro-Wilk -----		
p-value	0.13	0.34

TWF: Treated domestic wastewater plus mineral fertilizers; PWF: Potable water supplemented with mineral fertilizers; TW: Treated domestic wastewater; *: Significant (p-value<0.05); CV: Coefficient of variation (%).

The Tukey test results indicated that TWF (3.9 cm) did not differ significantly from PWF (3.8 cm) regarding fruit length per plant. However, TW (2.9 cm) had a significantly shorter fruit length per plant than the other treatments (± 4.59). The Shapiro-Wilk test confirmed that the residuals could be considered normal.

Concerning fruit diameter, TWF had an average of 2.85 cm per plant, which did not differ significantly from PWF (2.82 cm). However, TW (2.29 cm) differed considerably from the other treatments (± 3.81). The Shapiro-Wilk test indicated that the residuals could be considered normal. According to the Brazilian Program for Horticulture Modernization (PBMH) and Integrated Strawberry Production (PIMo) of 2009, strawberry fruits can be commercially classified into two classes based on their length and diameter: class 15 (1.5 to 3.5 cm) and class 35 (above 3.5 cm). Fruits from TW (2.9 cm) fall into class 15, while fruits from TWF (3.9 cm) and PWF (3.8 cm) can fall into class 35. However, due to nutritional deficiencies, fruits from TW cannot be commercialized despite falling into class 15. On the other hand, fruits from TWF and PWF are suitable for commercial purposes.

According to Passos and Trani (2013), the absence of nutrients such as nitrogen (N) and calcium (Ca) affects strawberry quality, leading to lower production, poor development, and fruit deterioration. The low concentration of mineral nutrients and high pH in fruits from TW may result in deformities and reduced size compared to TWF and PWF. However, these fruits can still be used for other purposes, such as making jams and candies. It is important to note that variations in strawberry fruit dimensions are influenced by genetic, environmental, nutritional, and physiological factors, as emphasized by Antunes et al. (2016).

ANOVA indicated a significant difference in the total soluble solids content (Fig. 6) between PWF, TWF, and TW. The Tukey test revealed that TWF (7.2) and PWF (6.9) were statistically similar to each other but differed significantly from TW (9.2). The Shapiro-Wilk test confirmed that the residuals could be considered normal, with a coefficient of variation between treatments of $\pm 10.04\%$.

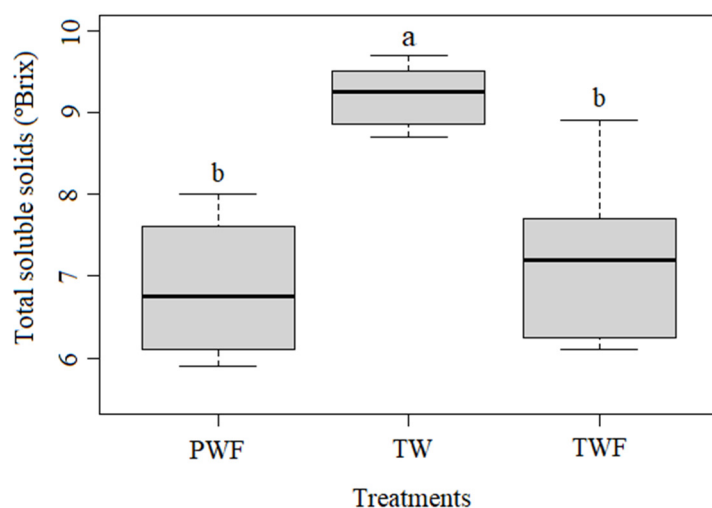


Fig. 6 Boxplot and Tukey test for averages of total soluble solids (°BRIX).

TWF: Treated domestic wastewater plus mineral fertilizers; PWF: Potable water supplemented with mineral fertilizers; TW: Treated domestic wastewater only. The means followed by the same letter do not differ from each other by Tukey's test at a 5% significance level.

Antunes et al. (2016) reported a °BRIX value of 6.96 for the San Andréas variety, indicating good fruit quality and sweetness. Franco et al. (2017) evaluated the same cultivar at different planting densities and obtained values ranging from 6.5 to 6.9. In our study, although no significant differences were observed in °BRIX, the averages obtained in TWF (7.2) and PWF (6.9) were similar to those reported by Antunes et al. (2016) and Franco et al. (2017). These findings suggest that strawberry cultivation in protected environments, along with proper fertigation, pH control, and plant care, can promote satisfactory °BRIX levels.

Despite the low mineral nutrient content and high pH in TW, which resulted in smaller fruit size and reduced production, the average °BRIX value obtained was relatively high at 9.2 °BRIX. These results are influenced by the production system, which affects the nutrient concentration and ultimately contributes to the production of smaller fruits with higher °BRIX contents. Schiavon et al. (2021) state that mineral nutrition influences the physicochemical properties of strawberries. Passos and Trani (2013) highlight that potassium affects the organoleptic characteristics of strawberries: aroma, flavor, and sugar content. They also state that calcium deficiency reduces productivity, worsening strawberry quality, and leads to low sugar levels. Thus, the differences in °BRIX levels found in this research may be related to the imbalance of nutrients provided by treated sewage (Table 2), and the calcium concentrations found did not affect the sugar levels produced. However, the lack of other nutrients negatively affected fruit size and production. Smaller fruits (22% on average, Table 6) observed in TW increased the °BRIX content but in stunted plants with a low production level and deformities.

Fruit microbiological analysis

The microbiological method used to analyze the strawberry fruits in this study follows the maximum standards set by Resolution No. 12 of the National Health Surveillance Agency, ANVISA (2001). According to this resolution, thermotolerant coliforms, including *E. coli*, should be absent in the analyzed samples (Table 7).

Table 7. Quantification of bacteria (*E. coli*) in strawberry fruits.

Treatment	<i>E. coli</i> (MPN)
TW	Absent
TWF	Absent
PWF	Absent

TW: Treated domestic wastewater only; TWF: Treated domestic wastewater plus mineral fertilizers; PWF: Potable water supplemented with mineral fertilizers; MPN - Most likely number.

According to Cuba et al. (2015), soilless cultivation systems function as barriers that prevent direct contact between the fertigated nutrient solution and plants, allowing nutrient absorption solely through the roots. Moreover, using plastic mulch on the upper part of the cultivation profile further enhances this barrier. Consequently, bacteria lack active structures to penetrate intact plant tissues (Reis and Olivares, 2006; Rhoden et al., 2019).

Despite the low concentration of *E. coli* bacteria in the nutrient solution of treatments TW and TWF, the microbiological analysis of the strawberry fruits revealed the absence of *E. coli*. According to Brazilian regulations, treated domestic wastewater is not permitted for vegetable cultivation. However, substrate cultivation systems employ it as a mitigating measure and allow for utilizing this wastewater in fertigation. Under Brazilian legislation, treated domestic sewage is not permitted in any vegetable cultivation, so parameters with minimum or maximum limits for microbiological issues are not discussed. On the other hand, the results obtained in this experiment, in which its application is used in a cultivation system with the substrate, in addition to being a mitigating measure, show that there is the possibility of exploring the potential of treated domestic sewage taking into account cultivation methods through fertigation. In future projects, the technique of applying treated domestic wastewater could be used on ornamental plants or native plant seedlings.

Conclusion

Our study demonstrates that using treated domestic wastewater supplemented with mineral fertilizers (TWF) as a source of water and nutrients effectively achieved high yields and quality fruits in strawberries compared to conventional potable water containing only mineral fertilizers (PWF). However, in the absence of mineral fertilizer supplementation, it did not result in commercial productivity and market-expected fruit qualities due to various abnormalities and deficiencies, including nutrient deficiencies in plants, significantly lower productivity, and inferior fruit quality compared to treatments receiving mineral fertilizer.

The productivity and quality of the cultivar San Andréas, including fruit number, length, diameter, fresh weight, and total soluble solids, were statistically similar between the TWF method and the conventional cultivation system (PWF). The trough culture system acted as a physical barrier, preventing direct contact between the treated domestic wastewater and strawberry fruits. This minimized the risk of fruit contamination by bacteria and other potential pathogens, such as *E. coli*.

Fertigation using treated domestic wastewater proved to be an environmentally sustainable and economically viable alternative, leading to a 13.1% reduction in mineral fertilizer usage and saving approximately 4,500 L of potable water throughout the cultivation process.

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