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# Decay of pathogens (indicators of *Escherichia coli* and *Salmonella spp*.) in soil due to the application of reuse water

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## ABSTRACT

Reuse water is defined as the reuse of water from treated effluents, it requires careful monitoring to avoid damage to environmental health. This study evaluates the decay of *Escherichia coli* and *Salmonella spp*. bacteria in soil irrigated with reused water for agricultural reuse, without damaging public or environmental health. The decay of *Escherichia coli* and of Salmonella spp. was verified using the Filter Membrane method SS AGAR culture medium was used. The decay curves over time were made using the Sigmaplot program. Each experimental group had 4 pots (one group irrigated with recycled water and the other with drinking water), two pots containing vegetation cover and two containing only soil. In crops irrigated with reused water, the survival time of *Salmonella spp*. was double compared to the others, and *E. coli* survival did not vary between groups. Pots with bare soil irrigated with uncontaminated reused water showed a faster decline in *Salmonella spp*. For agricultural reuse, irrigation must be done by drip and with the use of personal protective equipment. It is essential to create national legislation to protect public and environmental health.

**Keywords:** agricultural reuse, decay analysis of microorganisms, public health.

# Decaimento de patógenos (indicadores de *Escherichia coli* e *Salmonella spp*.) no solo devido a utilização de água de reúso

## RESUMO

A água de reúso é definida como a reutilização de águas provenientes de efluentes tratados, e requer segurança sanitária para evitar danos à saúde ambiental. O objetivo foi avaliar o decaimento de bactérias (*Escherichia coli* e *Salmonella* spp.) em solo irrigado com água de reúso, visando o reúso agrícola sem causar danos à saúde pública e ambiental.O decaimento de *Escherichia coli* foi verificado utilizando o método de Membrana Filtrante, e o de Salmonella spp. foi utilizado o meio de cultura SS AGAR. As curvas de decaimento ao longo do tempo foram feitas pelo programa Sigmaplot. Cada grupo experimental tinha 4 vasos (grupo irrigado

com água de reúso e outro com água potável) sendo dois vasos contendo cobertura vegetal e dois contendo apenas solo. Nos cultivos irrigados com água de reúso, o tempo de sobrevivência de *Salmonella* spp. foi o dobro em comparação aos demais, e a sobrevivência da *Escherichia coli* não variou entre os grupos. Os vasos com solo nu irrigados com água de reúso sem contaminação mostraram um decaimento da *Salmonella* spp. mais rápido. Para o reúso agrícola a rega deve ser por gotejamento e com a utilização de equipamentos de proteção individual. É fundamental a criação de legislação nacional, prevenindo danos à saúde pública e ambiental.

**Palavras-chave:** análise de decaimento de microrganismos, reúso agrícola, saúde coletiva.

# 1. INTRODUCTION

Approximately 45% of Brazilian sewage is released into water bodies without adequate treatment, and every day on average 9.1 thousand tons of sewage are generated, compromising the sanitary quality of more than 110 thousand km of extension of water sources (ANA, 2022). According to the 2030 Agenda for Sustainable Development of the United Nations Development Program, water scarcity affects more than 40% of the world's population, and is expected to increase due to climate change and the lack of adequate water management. Goal Number 6 of the sustainable development goals (SDGs), Drinking Water and Sanitation, is to ensure the availability and sustainable management of water and sanitation for all. It is possible to achieve this objective through international cooperation, protection of springs, rivers and basins, and the sharing of water-treatment technologies (Palma, 2017). Amid the scarcity of water for human consumption, drought in different regions, inequality in access to water with sanitary quality in different regions of the world, reused water has been studied as an alternative and sustainable source for society. Furthermore, it has become an important option, as it can reduce the amount of waste by releasing treated sewage into water bodies, allowing for a more rational use of water resources, and being an alternative source of available water (Martínez *et al*., 2013).

Currently, in Brazil and worldwide, there are several examples of the use of reuse water in agriculture (Handam *et al*., 2022a; Moura *et al*., 2019; Mancuso and Santos, 2013). Reuse water is defined as the reuse of water, specifically treated effluents (Moura *et al*., 2020; Morais *et al*., 2016). It can be classified according to Moura *et al*. (2020), who conceptualized the origin of reuse water as follows: (i) Local or internal reuse, the water reuse obtained from greywater treatment from residential reuse and reuse of new ventures; (ii) External reuse, from black waters that pass through a sewage treatment plant and from reused water (ETE + WWTP).

Agricultural activity consumes the most fresh water in the world, around 70% in Brazil, (FAO, 2017). Three quarters of the total water withdrawn globally is allocated to agricultural activities (UN, 2021). However, the scarcity of water sources for this activity in various regions makes reuse water an attractive alternative to address this problem (Moura *et al*., 2020). This alternative brings several benefits to agriculture, such as essential nutrients for vegetables, including nitrogen, phosphorus and potassium; promoting plant growth (increased agricultural production); and reducing the use of artificial fertilizers (USEPA, 2012). However, it is essential to ensure sanitary quality to avoid harm to human health and the environment. This type of water may contain micropollutants such as chemicals and microorganisms, which can pose risks to public health and the environment (Handam *et al*., 2021a). The use of reuse water in the soil microbiota may benefit or not benefit microorganisms, depending on the amount applied and the composition of the reuse water. When applied in high quantities and with a high nutrient load such as phosphorus and nitrogen, pathogens, heavy metals, and antibiotics, the soil is unable to perform its recycling function, which can alter soil characteristics (Liu *et al*., 2013).

In Brazil, there is currently no federal legislation that establishes the quality parameters for the sanitary assessment of reused water as a water source, creating challenges in its application. For the production of reused water for agriculture, a federal law or regulation is essential in Brazil, defining forms of treatment and quality parameters, both bacteriological and physical-chemical, so that throughout Brazilian territory governments have the obligation to comply (Handam *et al*., 2021b).

In the environment, microorganisms suffer from conditions that are not always favorable to them, which leads to natural decay. Several conditions promote bacterial decay in the soil, such as solar radiation, temperature, physicochemical soil conditions, toxicity from other bacteria, and predation. These conditions vary depending on the climate of each location and favor the natural decay of bacteria, especially temperature and solar radiation (Alegbeleye and Sant'ana, 2020). Therefore, studies on bacterial decay in the soil are essential to understand how the modification of the soil microbiota occurs and the risks associated with the introduction of contaminants and pollutants that can alter environmental characteristics. Decay analysis aims to assess the lifespan of microorganisms in the soil matrix. The survival conditions of microorganisms vary depending on certain factors and external stimuli in the environment they inhabit (Alegbeleye and Sant'ana, 2020). This study therefore evaluates the decay of bacteria (*Escherichia coli* and *Salmonella spp*.) in soil irrigated with reused water, aiming for agricultural reuse without damaging public or environmental health.

## 2. MATERIAL AND METHODS

This study used a sample of reused water from a Wastewater Treatment Plant (WWTP). The water was chlorinated inside a tanker truck by sanitation company operators for water reuse, using a 0,5 ng/L ratio of hypochlorite. The water originated from the city of Rio de Janeiro, RJ. As an experimental control for comparison, potable water distributed by the sanitation company was used, sourced from a water treatment plant. The potable water underwent a dechlorination process in the laboratory (48 hours of resting in an open container). The "chlorinated" reused water sample was used to carry out this stage of the study, as it is the type most likely to be commercialized and destined for agriculture, and the one we had the easiest time obtaining in large quantities and fresh volumes for carrying out of experiments, in case we needed to repeat tests.

Approximately 5 liters of reused water were collected in a properly treated polypropylene container. The treatment process involved washing with 5% Extran soap followed by 10 rinses to ensure complete soap removal. Subsequently, rinsing was performed with analytical-grade acetone, followed by 5 rinses with deionized water, and then rinsing with analytical-grade ethanol, followed by 3 rinses with deionized water. The collected water was transported to the laboratory in a refrigerated box, accompanied by an ice pack to ensure better preservation.

For this work, a specifically constructed system was used for plant cultivation on laboratory benches, with controlled temperature (24°C) and lighting. Irrigation was achieved through directed dripping into the pots (Handam *et al*., 2022b). Sterilized glass pots were utilized, each having a small opening at the bottom for water drainage (irrigation water). Each pot was filled with 320 g of soil from the same supplier in Inhauma, Minas Gerais, MG. The dripping rate was controlled to provide 10 mL of water irrigation throughout the day, ensuring the soil remained moist.

For the decay analysis of *Escherichia coli* and *Salmonella spp.* pathogens (De Faria, 2015; Pereira *et al*., 2014), reference strains from accredited collections were obtained: *Escherichia coli* strain (Escherichia coli, INCQS 00178, CDC H34) and *Salmonella spp.* strain (*Salmonella enterica* subsp. Entérica, INCQS 00236, BM/NIH-T). These strains were provided by the Reference Microorganism Collection for Sanitary Surveillance-CMRVS, FIOCRUZ-INCQS, Rio de Janeiro, RJ. Each experimental group (irrigated with reused water and irrigated with

potable water) underwent testing under two conditions: one with vegetative cover (*Petroselinum crispum* – parsley, fully developed), and the other with uncovered soil (reused water group and control).

Decay analysis of pathogens was performed, evaluating the indicators *Escherichia coli* and *Salmonella spp.* (De Faria, 2015). To ensure the presence of a known load of *Escherichia coli* and *Salmonella spp*. in the reused water sample, assuming that reused water may also contain these contaminants, pathogens were introduced in both the experimental and control groups. For five days, a supplement of exogenous microorganisms (*Escherichia coli* and *Salmonella spp.)* was added to the soil in each pot, originating from reused water without artificial contamination and reused water with artificial contamination, as well as potable water with its own microbiota and potable water with artificial contamination. The procedure was conducted using external pipetting with a known load of 5 CFU/mL, totaling 25 CFU/mL of microorganisms in each pot: two pots in the reused water group, one with plants, and the other with soil only, and two pots in the control group, one with plants, and the other with soil only.

The crops were irrigated, over these five days, as follows:

1) cultivation irrigated with reused water with artificial contamination;

2) cultivation irrigated with reused water without artificial contamination, that is, with its own microbiota;

3) cultivation irrigated with drinking water with artificial contamination (control condition);

4) irrigated with drinking water without artificial contamination (control condition).

On the first day of decay monitoring (after the supplementation period, on the 8th day [T=0]), irrigation was carried out with 70 mL of distilled water in all pots, and the collection of irrigation water (water collected after passing through the soil following irrigation) was performed. Subsequently, soil samples were collected on the 1st, 2nd, 4th, 6th, 8th, 10th, 11th, 14th, 16th, 18th, and 20th weeks. The experiment was monitored for 140 days, with the pots being watered with sterile distilled water via drip, so that there was no contamination of an external water source, with the source of air being ignored, which could also happen in the test group with water from reuse and in the control group with drinking water. The irrigation water samples were processed on the same day as the collections.

The methodology for verifying *Escherichia coli* followed the Merck Manual (2010), using the chromogenic culture medium Chromocult® Coliform Agar (Cat. No. 1.10426.0100/500 Merck). This was combined with the Membrane Filtration method described in Standard Methods for the Examination of Water and Wastewater (Sotero-Martins *et al*., 2017; Handam *et al*., 2018). For the analysis of each sample, 500-fold serial dilutions were made, as described in Sotero-Martins *et al.* (2017). The analysis of *Salmonella spp.* was performed using the SS AGAR (*Salmonella - Shigella)* culture medium, following the MicroMEDSS brand protocol, which is a selective medium for the growth of *Salmonella spp*. After the culture medium was dried in a petri dish, 1 mL of the irrigation water sample was spread using a Drigalsky loop, and placed in an oven for 48 hours.

The quantification of thermotolerant coliform colonies and *Salmonella spp*. was carried out by CFU/mL of irrigation water. Colony count data for E. coli and *Salmonella spp*. were entered into an Excel spreadsheet, considering the dilutions for statistical calculations. Decay curves for *Escherichia coli* and *Salmonella spp*. were generated over time using the Sigmaplot 10.0 software (Pereira *et al.,* 2014).

An acceptable level of *E.coli* of up to 23 UFC/mL was considered, which is the standard established in standard ABNT 13969 (ABNT, 1997), Class 4, which defines a standard for thermotolerant coliforms (*E. coli*) for reused water for agriculture. The standard value was converted to values in CFU/mL, according to Sotero-Martins *et al.* (2017), according to statistical data observed in the work of Gronewold and Wolpert (2008), so it was 23 CFU/mL.

For *Salmonella* spp., it was only acceptable when it was absent in the soil, in accordance with Normative Instruction No. 07 of 12/04/2016, from the Ministry of Agriculture, Livestock and Supply (Brasil, 2016; De Faria, 2015).

The Access to Genetic Heritage activity was registered in the SisGen under number AAC8F2F, in compliance with the provisions of Brazilian Law No. 13.123/2015.

## 3. RESULTS AND DISCUSSION

Regarding the results of the *Escherichia coli* and *Salmonella spp*. decay analysis in the soil, for the pot with the cultivation of *Petroselinum sativum* (parsley) irrigated with reused water with artificial contamination, the decay time for *E. coli* and *Salmonella spp*. was observed to be 106 and 91 days, respectively. In contrast, the cultivation irrigated with potable water (control) with artificial contamination had decay times for *E. coli* and *Salmonella spp.* of 106 and 35 days, respectively (Figure 1). This indicates that the survival time of *E. coli* was similar in both the pot irrigated with reused water and the one irrigated with potable water.

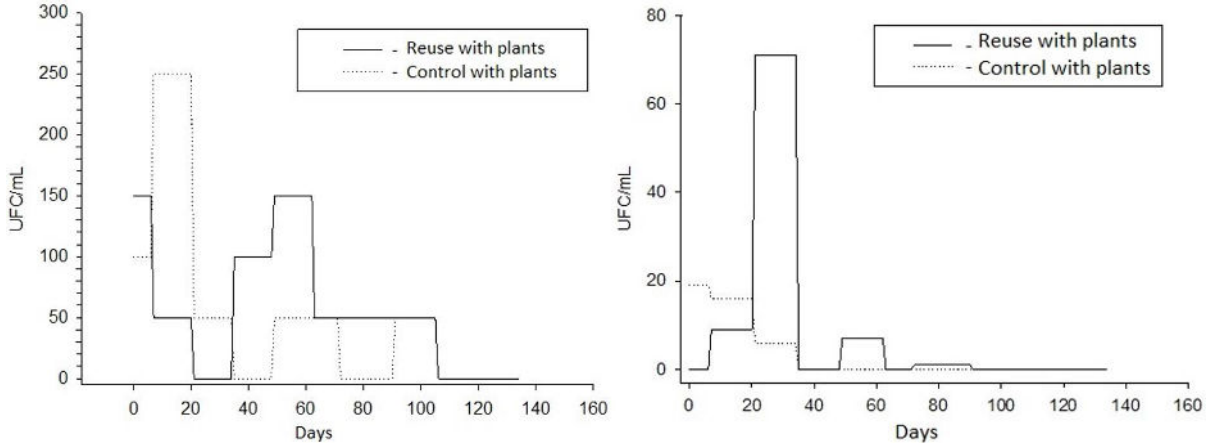


**Figure 1.** Result of the decay analysis of (A) *Escherichia coli* (CFU/ml) and (B). In plant crops with artificial supplementation of *E.Coli* and *Salmonella spp.*, irrigated with reuse water and potable water (control).

Regarding the pots with the cultivation of *Petroselinum sativum* (parsley) that did not have artificial contamination, in the pot irrigated with reused water, the decay time for *E. coli* and *Salmonella spp.* was found to be 120 and 106 days, respectively. On the other hand, for the cultivation irrigated with potable water, the decay time for *E. coli* and *Salmonella spp*. was 120 and 63 days, respectively (Figure 2).

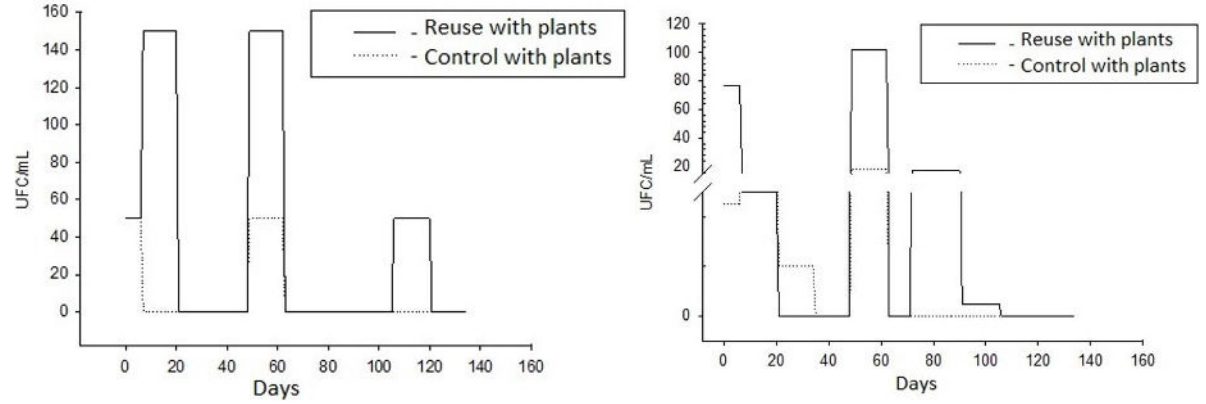


**Figure 2.** Result of the decay analysis of (A) *Escherichia coli* (CFU/mL) and (B) *Salmonella spp*. in plant crops, without supplementation, irrigated with reuse water and potable water (control).

It can be observed that the decay times of *Salmonella spp.* in the crops irrigated with reused water were longer compared to the crops irrigated with potable water. On average, the decay time in the reused water irrigated crops was 98 days (± 10), while in the pots irrigated with potable water, the average decay time was 49 days (± 19), which is twice the time. This demonstrates that reused water may contribute to the survival of the genus, as it may contain *Salmonella spp.* in its own microbiota. It is likely that reused water provides more nutrients to the soil, directly favoring the maintenance of the microbiota's survival. Therefore, due to this favorable environment for the maintenance of *Salmonella spp.* in the reused water matrix, it is essential for farmers to take precautions during irrigation to avoid primary contact with the skin and mucous membranes. The use of personal protective equipment, such as gloves, masks, and waterproof boots, is recommended to prevent contact with the soil irrigated with reused water, thereby avoiding health risks to the farmer handling the soil. Furthermore, based on the findings of this research and the literature review, irrigation using reused water should be carried out through drip irrigation, where water is directed straight to the soil and does not come into contact with the plant's leaves. Gatta *et al*. (2016) found that despite the identification of microorganisms such as *E. coli* and *Salmonella spp.* in sewage samples from secondary and tertiary treatment, artichoke crops irrigated with these samples were not contaminated. And the reduction of these bioindicators in the soil may be due to the drip irrigation system, which avoids water contact with the plant, and/or the death of bacteria in the soil, as well as the barrier through the plant roots.

Scherer *et al.* (2016) suggests that irrigating vegetables with water that has high levels of coliforms can contribute to the increase or maintenance of the population of microorganisms. This corroborates the study, as pots irrigated with reused water without artificial contamination had average *E. coli* concentration levels 1.8 times higher compared to the control group.

The survival time of the *E. coli* bacteria was longer compared to *Salmonella spp*., and there was no distinction regarding irrigation with potable water. On average, the decay time in pots irrigated with both reuse water and potable water was 113 days (± 9.8). De Faria *et al.* (2020) indicated that in agricultural soil cultivated with Eucalyptus containing fertilizers from sewage, it took 54 weeks, 379 days for this bioindicator pathogen to decay and return to the level of *E. coli* found in the soil, emphasizing that the survival time of *E. coli* also depends on the environmental temperature, being greater in tropical climates compared to temperate climates (De Faria *et al*., 2020). In temperate climates in Spain, Ngole *et al*. (2006) found *E. coli* decay after 90 days. In the present study, the cultures were at a controlled room temperature of 24ºC, with a tropical climate temperature, which favored a longer survival time for *E. coli*. A longer follow-up period for *E. coli* in the present study would be necessary; however, continuity in monitoring was not possible, due to the limited stock of the reused water sample collected, meaning it would not be possible to monitor the same batch of water. It is recommended that new studies with longer monitoring of the decay of E. coli be carried out.

Regarding the results of the decay analysis in the pot with soil only (uncovered), irrigated with reused water with artificial contamination, the decay time for *E. coli* and *Salmonella spp.* was observed to be 106 and 35 days, respectively. On the other hand, in the pot with uncovered soil irrigated with potable water with artificial contamination, the decay time for *E. coli* and *Salmonella spp.* was found to be 134 and 63 days, respectively (Figure 3).

In relation to the pots with uncovered soil that did not have artificial contamination, in the pot irrigated with reused water, the decay time for *E. coli* and *Salmonella spp.* was found to be 106 and 21 days, respectively. On the other hand, for the cultivation irrigated with potable water, the decay time for *E. coli* and *Salmonella spp.* was 134 and 21 days, respectively (Figure 4). This may be due to the greater incidence of direct light on the soil, as well as faster infiltration of water, which reduces the survival time of these microorganisms in the soil. Ultraviolet radiation is considered a toxic abiotic factor for microorganisms, and interferes with

their survival time (Thomas-Soccol *et al*., 2010). This shows that the cultivation of the vegetable *Petroselinum crispum* may also have resulted in the survival of *Salmonella spp*., as the vegetation cover shaded the soil, reducing the incidence of light passing directly to the soil.

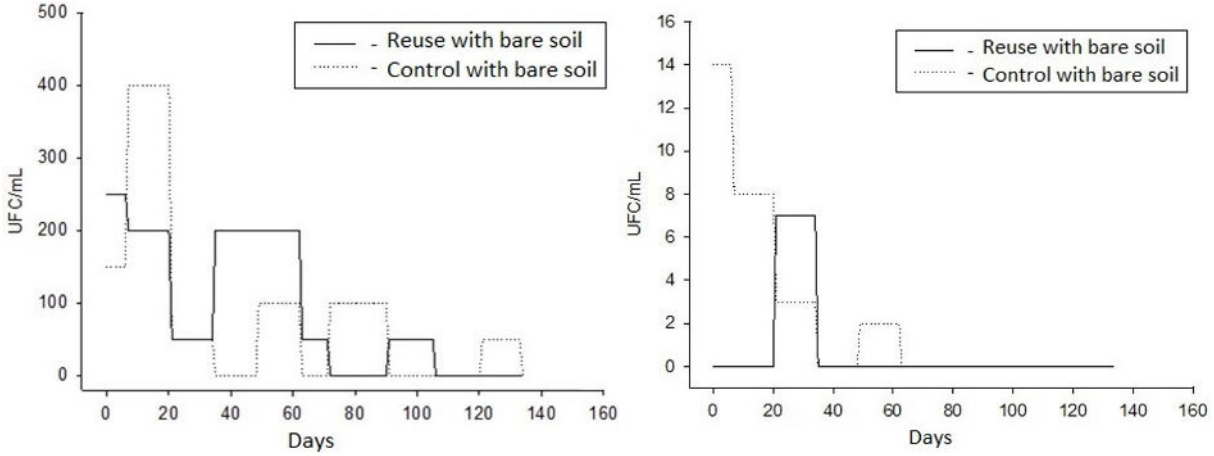


**Figure 3.** Result of the decay analysis of (A) *Escherichia coli* (CFU/mL) and (B) *Salmonella spp*. in the pots with uncovered soil, with artificial supplementation of *E. coli* and *Salmonella spp*., irrigated with reuse water and potable water (control).

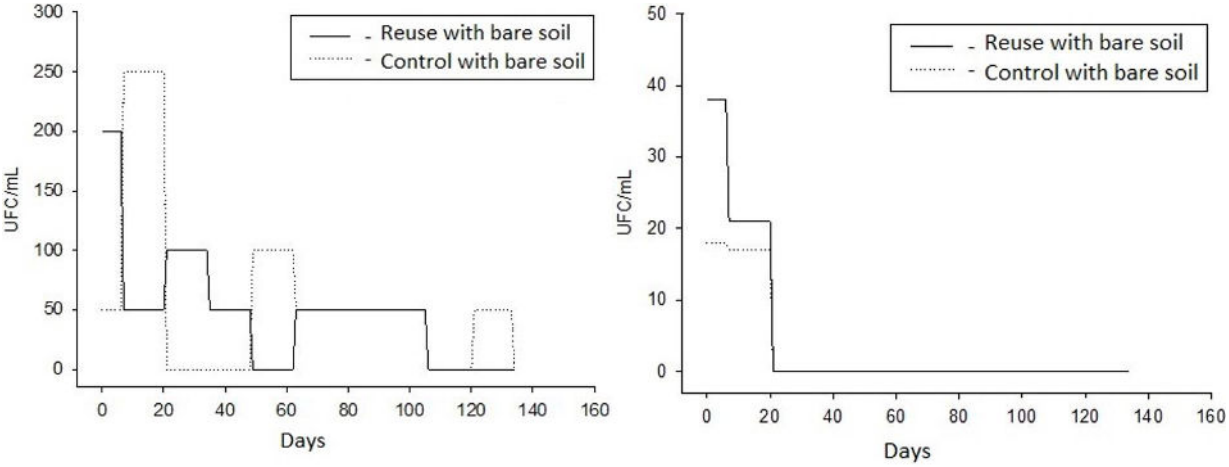


**Figure 4.** Result of the decay analysis of (A) *Escherichia coli* (CFU/mL) and (B) *Salmonella spp*. (CFU/ml) in the pots with uncovered soil, without supplementation, irrigated with reuse water and potable water (control).

On average, the decay time of *Salmonella spp*. in pots irrigated with reuse water was 28 days (± 9.8), while in pots irrigated with potable water, the average time was 42 days (± 29.6). The average decay time of *E. coli* in soils irrigated with reuse water was 106 days (± 0), while in soils irrigated with potable water, the average time was 134 days (± 0).

It can be observed that the decay time of *Salmonella spp*. in the pots with uncovered soil, which were not artificially contaminated, was faster compared to the pots with artificial contamination, with an average decay time of 21 days. The decay time of *Salmonella spp.* in the pots with uncovered soil was also shorter compared to the pots with the cultivation of *Petroselinum sativum* (parsley). This may be attributed to the higher incidence of direct sunlight on the soil, as well as water infiltration, which reduces the survival time of these microorganisms in the soil.

Zaleski *et al*. (2005) observed in a study a survival time of *Salmonella spp*. between two and three weeks in the soil. This aligns with the pots with uncovered soil without artificial contamination, which showed a decay time of 21 days. However, Thomaz-Soccol *et al*. (2010)

found that the survival time of *Salmonella spp*. in the soil can range from less than a week to up to six months, depending on the moisture and temperature conditions to which this microorganism is exposed. The WHO (2006) regulations indicate that the survival time of *Salmonella spp.* in the soil can be up to 70 days.

In the present study, the period of decline of *Salmonella spp*. may have been due to the direct incidence of radiation on the soil, which was not covered by cultivation. According to Alegbeleye and Sant'ana (2020), the decomposition of *Salmonella spp*. in the soil is strongly influenced by factors such as the incidence of sunlight, as well as other factors such as temperature, humidity and microbial activity.

In the pot with plants (Figure 4b) and the pot with uncovered soil, irrigated with reused water with artificial contamination, it was not possible to start the decay analysis from the first week as *Salmonella spp.* were absent. However, considering that the presence and increase of these microorganisms were observed in these pots in the second week, it was found that the decay was similar to that observed in the pots with cultivation compared to the pots with uncovered soil, occurring in 84 and 28 days, respectively, after their presence was detected.

In a study by De Faria (2015), peaks of increase and decrease in the count of *Salmonella spp*. over time were observed, as was also observed in most of the pots in the present study. Feitoza (2017), in their research, also identified significant variations in the presence of Salmonella, highlighting the emergence of peaks in the behavior of these bacteria.

The quantification of *Escherichia coli* bacteria in the parsley crops in the present study showed peaks of increase and decrease during the weeks studied after the start of the experiment. The peaks of *E. coli* increase can be justified, as suggested by Faria (2015), by the change in behavior of the genus when exposed to environmental factors. According to Mohammadi and Kashefipour (2020), the mortality of these microorganisms is directly related to factors such as temperature, humidity, pH, soil physical composition, and microbial competition. Thus, the controlled environmental factors in the laboratory over the crops may have favored the maintenance of *E. coli* in the soil. Scherer *et al.* (2016) suggests that irrigation of vegetables with water that has high levels of coliforms can contribute to the increase or maintenance of the microbial population. This is in line with the study, as the pots irrigated with reuse water had higher levels of *E. coli* compared to the control group.

Furthermore, plants are able to prevent the access of microorganisms in their capillaries (Agrios, 2004), as they absorb particles and solutes found in water, such as mineral salts with a size of 0.001 μm, while the dimensions of viruses, bacteria, are respectively 0.1 and 1 μm Therefore, they are more than 100 times larger than the size of the system used by the plants to absorb them, so the chance of contamination of food from the internal part of the plants is small. The greatest risk exists of the reused water coming into contact with the outside, of contaminating and polluting residues remaining on the leaves and stems (Schneider and Tsutiya, 2001). According to WHO (2006), irrigation techniques close to the ground, such as drip, reduce the chance of contamination of crops with pathogens that are in the soil, as there is no contact with the superficial aerial parts of the plants, such as the leaves and stems. However, farmers must be careful in direct contact with soil contaminated with microorganisms such as *E. coli* and *Salmonella* spp., as they can cause gastroenteritis. Therefore, for agricultural reuse, it is essential to use personal protective equipment, such as gloves, masks and waterproof boots, to prevent the farmer from coming into contact with soil irrigated with reused water.

In Brazil there is a challenge in the use of reused water, as there is currently no federal law that establishes sanitary quality parameters for evaluating agricultural reuse. For agricultural irrigation, it is essential that federal regulation be enacted that contains forms of treatment and sanitary quality parameters, both bacteriological and physical-chemical (Handam *et al*., 2021b). According to Moura *et al*. (2020), guidelines and programs containing definitions of the origins of reused water, as well as the forms of use and the sanitary quality parameters, must be drawn

up through federal legislation so that reused water can be used appropriately in agricultural Brazilian irrigation. Maia (2020) noted, “Regulation defines what is considered pollution, and it is up to the State to define parameters that are capable of preventing environmental degradation”. Federal regulation will allow each state to comply with national law and avoid damage to human health and the environment.

## 4. CONCLUSIONS

The results regarding the decay analysis of *Salmonella spp*. in *Petroselinum sativum* (parsley) crops demonstrate that the survival time of these microorganisms was double in crops irrigated with reused water compared to those irrigated with potable water. Reused water may be influencing the bacteria's maintenance by providing more nutrients, and it is likely that *Salmonella spp*. was present in the reused water sample, favoring the survival of the genus. On the other hand, the survival time of *E. coli* was higher compared to *Salmonella spp*. and did not vary with potable water irrigation. For agriculture reuse, further analyses should be done.

For the use of reused water in agriculture, the study showed that drip irrigation is recommended to avoid environmental damage and protect the health of farmers. It is recommended that irrigation using reused water be through drip application, where the water goes directly to the soil and does not come into contact with the leaves of the crops. During agricultural reuse, farmers should take precautions, and the use of personal protective equipment such as gloves, masks, and waterproof boots is advised to prevent contact with the soil irrigated with reused water and this type of water.

The results obtained from the pots with uncovered soil demonstrate that those irrigated with reused water without artificial contamination showed a faster decay time of *Salmonella spp.* compared to those irrigated with artificial contamination. The decay was also more accelerated compared to the pots with vegetative cover. This suggests that the presence of direct sunlight on the soil and water infiltration, due to the lack of vegetative cover, decreases the survival time of these microorganisms in the soil at a faster rate.

In order to guarantee the sanitary quality of reused water for safe use and prevent any negative impact on public health and the environment, it is crucial to establish national legislation for agricultural reuse that includes the source of this water, sanitary quality standards and methods of treatment for its production. This will allow each state to comply with the law at a national level and avoid any harm to human health or the environment.

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