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Vancomycin Degradation using UV/H_2O_2 in A Batch Reactor

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***Abstract*:- Micropollutants have been gaining prominence over the years for being increasingly more present in water and, consequently, our bodies can come into contact with these compounds. Micropollutants are toxic, mineral or organic substances in low concentrations, that persist even after conventional treatment, and which exhibit bioaccumulative properties if absorbed by any organism. Pharmaceutical drugs are among the many substances that form this class of compounds. Among these is vancomycin, a complex glycopeptide utilized in the treatment of bacterial infections. The degradation of these micropollutants demand more oxidative processes capable of breaking the bonds in these compounds and leaving them in the form of inactive minerals. With this in mind, the present work has the objective of evaluating the degradation of vancomycin over time under UV photolysis and the combination of UV and H_2O_2. For this, aqueous solutions of 200 mg/L of vancomycin were prepared and added to a batch reactor equipped with 2 germicidal lamps. Tests using both photolysis and UV in combination with 0.25 mg/L H_2O_2 were performed. Aliquots were collected every 15 minutes and the absorbance of the samples was checked in a UV-Vis spectrophotometer. Total treatment time for both tests was two hours. As results, the photolysis-only treatment was found to be insufficient for the degradation of vancomycin in solution. The combined UV/H_2O_2 treatment, however, was capable of degrading the samples up to 90% of their original concentration in two hours. Toxicity of treated solutions towards *Lactuca sativa* was evaluated, and found to be non-toxic. Therefore, we propose that the combined UV/H_2O_2 treatment comprises an effective option for the degradation of vancomycin and its elimination from supply waters, providing higher quality to its consumers.**

Keywords: Micropollutants; vancomycin; Advanced Oxidation Processes; toxicity; Lactuca sativa.

INTRODUCTION

In recent years, a new class of organic contaminants, known as “micropollutants” or “emerging pollutants”, has been detected in aquatic resources and has become a problem of great environmental concern (ARIAS et al., 2018; CHEN et al., 2021). This class of contaminants is comprised of chemical compounds such as pesticides, pharmaceutical products, endocrine disruptors, and personal care products. Micropollutants exhibit recalcitrant properties, accumulating in the environment even after conventional treatment. Given their complexity, their implications on the human organism are not completely understood, exacerbating the necessity for better, more efficient treatment options (ARIAS et al., 2018). One of the main points of entry of micropollutants to the environment are secondary effluents, which originate from wastewater treatment plants. Despite appearing in relatively low concentrations, they must be properly removed due to their presumed harmful effects on human beings and the environment (SUGIYONO; DEWANCKER, 2020).

Among the aquatic contaminants of pharmacological origin, around 15% of the ones found in the environment belong to the antibiotic class, of which liquid effluents comprise the main source of contamination (BALAKRISHNA et al., 2017). The first organisms to be affected by the contamination with antibiotics are aquatic organisms, as they are continuously under exposure (ARIAS et al., 2018). In environmental matrices, antibiotics have been detected in increasing amounts: in superficial waters, the levels found are still considered moderate (0,0002 – 100 $\mu g.L^{-1}$), while higher levels have been found in hospital wastewater (0,003 – 101 $\mu g.L^{-1}$) and in the effluent originating from pharmaceutical industries (0,026 – 43900 $\mu g.L^{-1}$) (DAVIES *et al.*, 2021; SANTOS *et al.*, 2010). Because antibiotics possess specific structures and properties, their removal is more complicated. The problem is also exacerbated because antibiotics are present in several environmental matrices of different characteristics each (RAM; KUMAR, 2020).

Antibiotics are pseudo-persistent, bioaccumulative, and biologically active contaminants, that is, they were introduced with the intent of producing effects on microorganisms (HERNANDO et al., 2006). Their introduction into the environment is defined by the physicochemical properties of each antibiotic (KEMPER, 2008). These compounds can be responsible for the presence of resistant microorganisms and other problems of public health concerns (ANDRÉS-COSTA; ANDREU; PICÓ, 2017; CHOI et al., 2018), according to studies stating that antibiotics can cause allergies and general sensibilities (KÜMMERER, 2009).

One of the antibiotics found in aquatic environments is vancomycin ($C_{66}H_{75}C_{l2}N_9O_{24}$), a complex glycopeptide used in the treatment of bacterial infections, specifically against Gram-positive bacteria (MOELLERING, 2006). Developed in the 1950s, vancomycin is produced via fermentation by the actinobacterium *Amycolatopsis orientalis*. The molecule has a complex structure containing sugars and amino acids, resembling the structure of the bacterial cell wall, thus capable of interfering with and disrupting it (ANTONOPLIS et al., 2018).

One of the most promising methods for the treatment of contaminated waters are advanced oxidation processes (AOPs), the purpose of which is to degrade the more complex organic molecules into less aggressive chemical substances, through the formation of hydroxyl radicals (OH^-), chemical alterations on the substrate, and possibly the complete mineralization (GHANBARI; MORADI, 2017; JI *et al.*, 2021).

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Ultraviolet (UV) radiation, oxidation by hydrogen peroxide (H_2O_2), and the combination of these processes (UV/H_2O_2), can actively eliminate some emerging contaminants from wastewaters (RIZZO *et al.*, 2019). The hydroxyl radicals (OH^-) generated by the combination of UV/H_2O_2 are capable of breaking the chemical bonds of several emerging contaminants that persist after treatment under UV radiation (K. ALHARBI *et al.*, 2017). As such, the usage of UV light with a catalyst has been referred to considerably degrade pesticides and pharmaceutical products in wastewater, although its full-scale application is still restricted (HADEI *et al.*, 2021; KUMAR; TRAVAS-SEJDIC; PADHYE, 2020).

Thus, the goal of this work was to propose an efficient treatment for the elimination of vancomycin from supply waters, through UV radiation alone and the combination of UV and H_2O_2. The effects of treatment with AOP on the germination and growth of seeds of *Lactuca sativa* (lettuce) was evaluated. *L. sativa* was chosen because it is considered a standard species suitable for ecotoxicological studies covering terrestrial plants due to its low cost, simplicity, and ability to react quickly to exposure to chemical substances (BRAGANÇA *et al.*, 2018; OECD, 2006).

METHODOLOGICAL PROCEDURES

Vancomycin (Antibióticos do Brasil, lot: 109480C) solutions (200 mg/L) were prepared and the most appropriate wavelength was evaluated for the preparation of the calibration curve in a UV-Visible molecular absorption spectrophotometer (Genesys 10S, ThermoScientific). Afterwards, the calibration curve was prepared using the concentration points of 12.5, 25, 50, 100, and 200 mg/L.

Degradation tests were first performed using only UV radiation over the solutions. Assays were performed in a reactor (Figure 1) composed of two 15W UV lamps in a closed cylindrical support of polyvinyl chloride (PVC), into which 100 mL of the 200 mg/L vancomycin solutions were added. The combination of UV radiation and various concentrations of H_2O_2 was also tested. For these conditions, different amounts of H_2O_2 (0.10, 0.25, and 0.50 mL) were added to each solution that was inserted in the reactor. These tests were based on the methodologies of Cibati et al. (2021) and Hong et al. (2022).

For both sets of experiments, aliquots of 3 mL were collected every 15 minutes and their absorbance was checked in a spectrophotometer. The total irradiation time was determined according to when readings had stabilized.

Figure 1 – Reactor for bench testing of advanced oxidation processes

For the toxicity tests, lettuce (*L. sativa*) seeds and Petri dishes with filter paper were used. Positive control (distilled water), negative control (potassium dichromate solutions, $K_2Cr_2O_7$, to prevent growth) and five treated solutions were used on the plates: non-irradiated 200 mg/L vancomycin solution, and irradiated vancomycin solutions containing H_2O_2, diluted in distilled water to 100%, 75%, 50%, and 25% of the original concentration. Tests were performed in quadruplicates, at a temperature of 22 ºC for 96 hours. Seeds were selected and 5 seeds were added to each Petri dish. The volume of solution used in the plates was 1.5 mL.

In order to examine the results, the methodology of Tam & Tiquia (1994) was applied, in which the relative seed germination (RG) (Equation 1) and relative root growth (RRG) (Equation 2) data were used to determine the germination index (GI) (Equation 3).

$$RG\ (\%) = \frac{N\ of\ germinated\ seeds\ in\ treated\ solution}{N\ of\ germinated\ seeds\ in\ control\ solution}\ x\ 100 \quad (1)$$

$$RRG\ (\%) = \frac{Average\ root\ growth\ in\ treated\ solution}{Average\ root\ growth\ in\ control\ solution}\ x\ 100 \quad (2)$$

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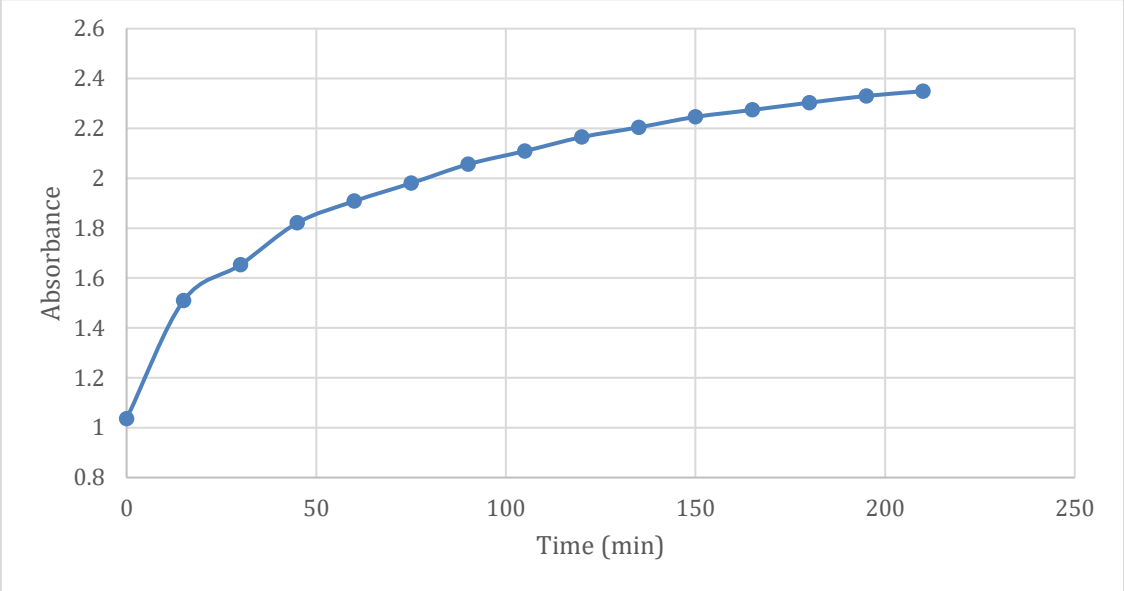
$$GI\ (\%) = \frac{RG\ (\%)\ x\ RRG\ (\%)}{100} \quad (3)$$

Toxicity was evaluated by observing the number of germinated seeds in each solution and evaluating root growth on the plates.

RESULTS AND DISCUSSION

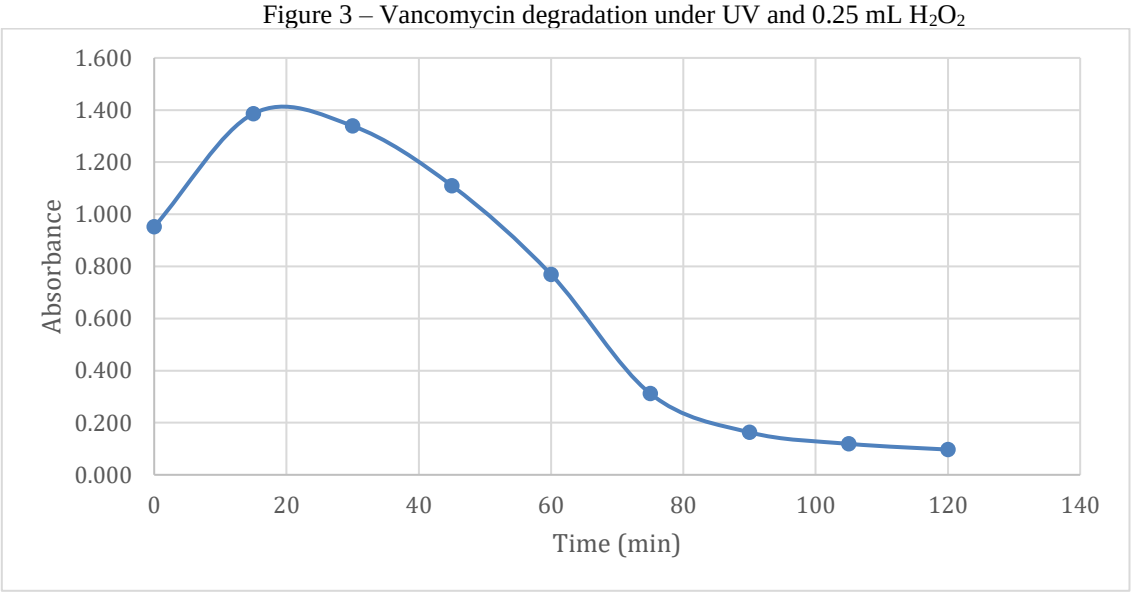
Little to no degradation could be observed in the vancomycin solutions treated with UV radiation alone. As shown in Figure 2, absorbance readings increased with UV light exposure, and stabilized after around 3 hours of treatment.

Figure 2 – Degradation of vancomycin under UV radiation



The treatment using UV/H_2O_2, on the other hand, had the best result when 0.25 mL of H_2O_2 were added to the vancomycin stock solutions. After 2 hours of irradiation, the spectrophotometer readings stabilized near zero absorbance, as shown in Figure 3.

Figure 3 – Vancomycin degradation under UV and 0.25 mL H_2O_2



Still according to Figure 3, it is possible to observe that absorbance increased in the first 20 minutes, followed by a decrease of approximately 90% in the following hours. Similar observations were made in the study by Hong et al. (2022), which investigated the mechanism behind propiconazole degradation under photolysis and the combined UV/H_2O_2 process, where an increase in toxicity was observed for the first 10 minutes, but decreased and was eventually eliminated as reaction time moved forward. Thus, the UV/H_2O_2 process was found to be an effective treatment for the removal of propiconazole in water.

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Khorsandi et al. (2019) evaluated the degradation and mineralization efficiency of treatments using photolysis and UV/H_2O_2 on solutions containing ceftriaxone in concentrations of 5, 10, and 20 mg/L. After 120 minutes under treatment with UV/H_2O_2, a removal rate of 100% was observed. In the photolysis assays, 61% of ceftriaxone was degraded in the same period of time.

Michael (2020), on the other hand, evaluated the UV/H_2O_2 treatment of solutions of 5mg/L ciprofloxacin and sulfamethoxazole, and concluded that these conditions were able to eliminate the antibiotics after 90 minutes of irradiation.

As for the toxicity tests after UV/H_2O_2 treatment, no seed growth could be observed in the negative control ($K_2Cr_2O_7$), while positive control exhibited the highest rates of germination and root growth, as show in in Table 1.

Table 1 – Average germination and root growth of *L. sativa*

Solution	Average germination (seeds)	Average root growth (cm)
$K_2Cr_2O_7$	0.00 ± 0.00	0.00 ± 0.00
Vancomycin 200 mg/L	3.75 ± 0.83	1.30 ± 0.41
Distilled water	4.75 ± 0.43	2.38 ± 0.41
100% degraded	4.00 ± 0.71	1.58 ± 0.38
75% degraded	4.00 ± 0.00	1.63 ± 0.30
50% degraded	4.50 ± 0.50	1.88 ± 0.22
25% degraded	4.75 ± 0.43	1.76 ± 0.18

In the treatments with UV/H_2O_2 irradiation, the average germination, average root growth, and germination index increased as the concentration of vancomycin in the treated solutions decreased. The 200 mg/L, non-irradiated vancomycin solution, on the other hand, showed lower averages than the other conditions.

Table 2 – Results for RG, RRG, and GI of *L. sativa*.

Solution	Relative seed germination (%)	Relative root growth (%)	Germination Index (%)
Vancomycin 200 mg/L	78.95	54.62	43.12
100% degraded	84.21	55.90	55.90
75% degraded	84.21	68.49	57.67
50% degraded	94.74	78.99	74.83
25% degraded	100.00	73.95	73.95
Distilled water	100.00	100.00	100.00

Figure 4 – Relative germination of lettuce *(L. sativa)* seeds.

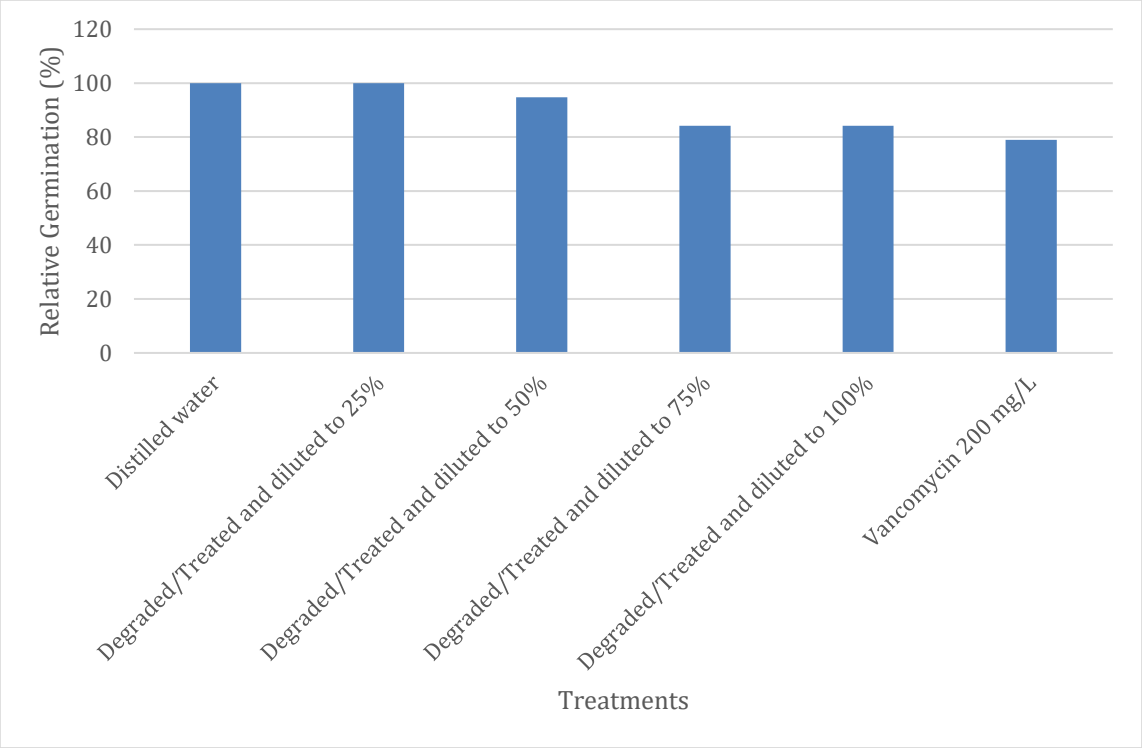

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Figure 5 – Relative growth of *L. sativa* roots

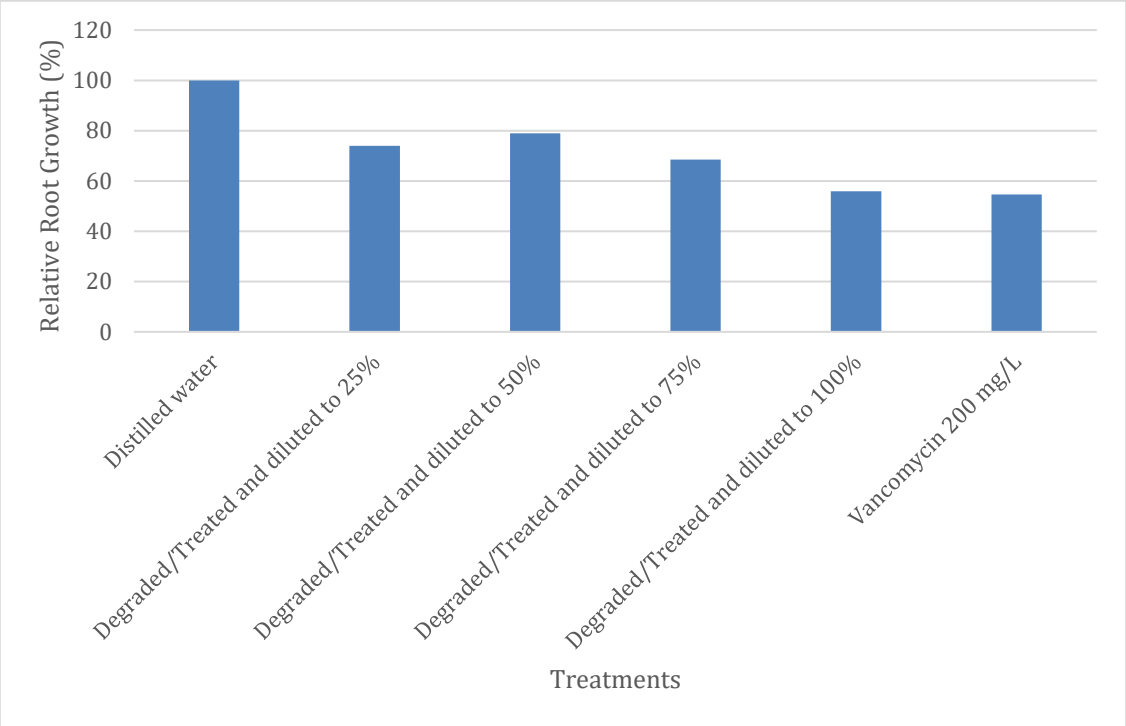
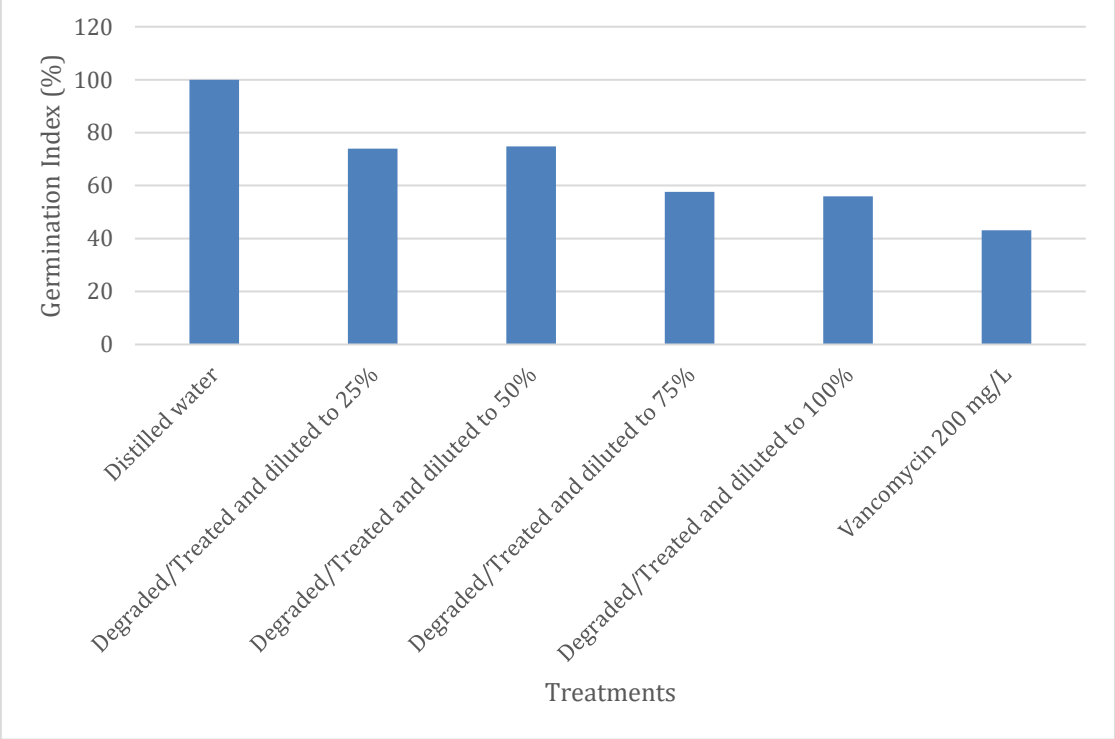


Figure 6 – Germination Index



As per Figures 4, 5, and 6, it is possible to identify the increasing growth in germination and root growth rates as the treated solutions were diluted. As the curve decreases, it is possible to evaluate that, as the concentration increases, the germination index is lower, making the solution more toxic to the test organism.

According to Cesaro et al. (2015), GI values between 0 and 40% indicate severely inhibited germination, while values between 40 and 80% are considered to indicate a mild inhibition. Values between 80 and 120% are considered non-significant. Therefore, in the present work, the inhibition of the Germination Index was considered mild.

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CONCLUSION

It was concluded that photolysis alone was not effective enough for the degradation of vancomycin in solution, and resulted in higher absorbance in comparison to the original solution. However, the combination of UV photolysis with 0.25 mg/L H_2O_2 for a period of 2 hours resulted in efficient degradation of vancomycin, observed through a decrease in absorbance of the samples. The toxicity tests showed positive results for germination, and thus the solutions were not considered toxic to the test organism *L. sativa*. Therefore, it has been possible to show a safe and adequate treatment for the degradation of the drug, ensuring a better quality of water for its consumers.

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