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## Article

# A Study of Treatment of Reactive Red 45 Dye by Advanced Oxidation Processes and Toxicity Evaluation Using Bioassays

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**Abstract:** Advanced oxidation processes (AOPs) hold great promise to degrade and detoxify industrial-based effluents. The Reactive Red 45 dye aqueous solutions were treated with AOP using UV and gamma radiation alone and then in the presence of H<sub>2</sub>O<sub>2</sub>. The dye initial concentration, UV exposure time, and gamma-ray absorbed dose were optimized for maximum degradation. The degradation of dye was 88.85% and 77.7% using UV/H<sub>2</sub>O<sub>2</sub> (1 mL/L) at a UV exposure time of 180 min for 50 mg/L and 100 mg/L, respectively. The degradation was noted as 100% and 93.82% as the solutions were subjected to a gamma/H<sub>2</sub>O<sub>2</sub> (1 mL/L) absorbed dose of 2 kGy. The chemical oxygen demand was reduced to 77% and 85% by treating the dye samples with UV/H<sub>2</sub>O<sub>2</sub> and gamma/H<sub>2</sub>O<sub>2</sub>, respectively. The removal efficiency (G-value), dose constant (k), D<sub>0.50</sub>, D<sub>0.90</sub>, and D<sub>0.99</sub> for gamma-irradiated samples were also calculated. The reduction in toxicity for treated samples was monitored by using the *Allium cepa*, Hemolytic, and brine shrimp (*Artemia salina*) tests while the Ames test was performed for mutagenic assessment. The *A. cepa* test showed 39.13%, 36.36%, and 47.82% increases in root length (RL), root count (RC), and mitotic index (MI), respectively, in UV/H<sub>2</sub>O<sub>2</sub>-treated samples while 48.78%, 48.14%, and 57.14% increases were shown with gamma-ray in conjunction with H<sub>2</sub>O<sub>2</sub>. The hemolytic test showed 21.25% and 23.21% hemolysis after UV/H<sub>2</sub>O<sub>2</sub> and gamma/H<sub>2</sub>O<sub>2</sub> treatments, respectively. The brine shrimp (*Artemia salina*) test showed 84.09% and 90.90% decreases in the nauplii death after UV/H<sub>2</sub>O<sub>2</sub> and gamma/H<sub>2</sub>O<sub>2</sub> treatments, respectively. The mutagenicity of UV/H<sub>2</sub>O<sub>2</sub>-treated solutions was reduced up to 84.41% and 77.87%, while it was 87.83% and 80.88% using gamma/H<sub>2</sub>O<sub>2</sub> using TA98 and TA100 bacterial strains, respectively. The advanced oxidation processes based on UV and gamma radiation in conjunction with H<sub>2</sub>O<sub>2</sub> can be applied for the degradation and detoxification of textile waste effluents efficiently.

**Keywords:** gamma radiation; UV radiation; cytotoxicity; Reactive Red 45 dye; advanced oxidation process



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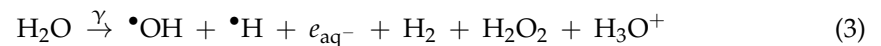
## 1. Introduction

Dyes are chemically stable compounds and are a serious problem when dealing with textile waste effluent. Being highly soluble and environmentally problematic compounds, reactive dyes are the most discussed dyes in the literature [1]. Textile waste effluent reduces water transparency and sunlight penetration which affect aquatic life when mixed with ground and surface waters [2]. The adverse pollution effects of dyes in water systems require treatment of samples before being discharged into any water bodies [3]. Physical

methods do not remove the dyes from the water completely and may convert them into a secondary pollutant. The chemical oxidation method using hydrogen peroxide, potassium permanganate, chlorine, ozone, etc., to remove pollutants also has some limitations as it is a time-consuming process [4]. The AOP is an effective method that has gained more attention in decontaminating water-containing dyes [5]. During AOP, a strong oxidizing species ( $\bullet\text{OH}$ ) is produced which converts the toxic organic compounds into simple and less toxic compounds via a chain reaction [6]. The AOP is advantageous to other treatment methods as it is reliable, easy to handle, reduces manpower, and has the potential to degrade and detoxify the organic matter without producing a secondary pollutant [7]. Various AOPs based on ozonation, perozone, UV/O<sub>3</sub>, UV/H<sub>2</sub>O<sub>2</sub>, gamma/H<sub>2</sub>O<sub>2</sub>, UV/TiO<sub>2</sub>, and UV/ZnO have been employed to degrade textile waste effluents [5–8]. UV radiation in the presence of H<sub>2</sub>O<sub>2</sub> can decompose the dye molecules effectively according to the following Equations (1) and (2) [9].



Gamma radiation along with H<sub>2</sub>O<sub>2</sub> is believed to be an efficient technique to decompose organic matter. H<sub>2</sub>O<sub>2</sub> is a strong oxidizing agent that promotes the AOP by producing hydroxyl radicals ( $\bullet\text{OH}$ ), which not only scavenge the species  $\bullet\text{H}$  and  $e_{\text{aq}}^-$  produced as a result of radiolysis of water but also enhance the production of  $\bullet\text{OH}$  (Equations (3)–(5)), which is needed to enhance the degradation rate [10,11].



To avoid the generation of toxic compounds, biological safety is an important measure when using radiation to degrade wastewater [12–17]. The bioassays including *A. cepa*, hemolytic, and brine shrimp (*Artemia salina*) tests are used as quick and reliable methods. The mutagenic behavior of the probe compound can effectively be measured by the Ames test [18–20].

This study was designed to treat Reactive Red 45 dye solutions by AOPs using UV and gamma radiation along with H<sub>2</sub>O<sub>2</sub>. The effect of radiation on pH, COD, and degradation was monitored. The G-value, k, D<sub>0.50</sub>, D<sub>0.90</sub>, and D<sub>0.99</sub> of gamma-irradiated samples were investigated. The *A. cepa*, hemolytic, and brine shrimp (*Artemia salina*) tests were used for toxicity assessment, whereas the mutagenicity evaluation was carried out using the Ames test.

## 2. Materials and Methods

### 2.1. Chemicals

The Reactive Red 45 dye was purchased from the local market and was used without prior purification. The characteristics of the dye are given in Table 1 and its structure is shown in Figure 1.

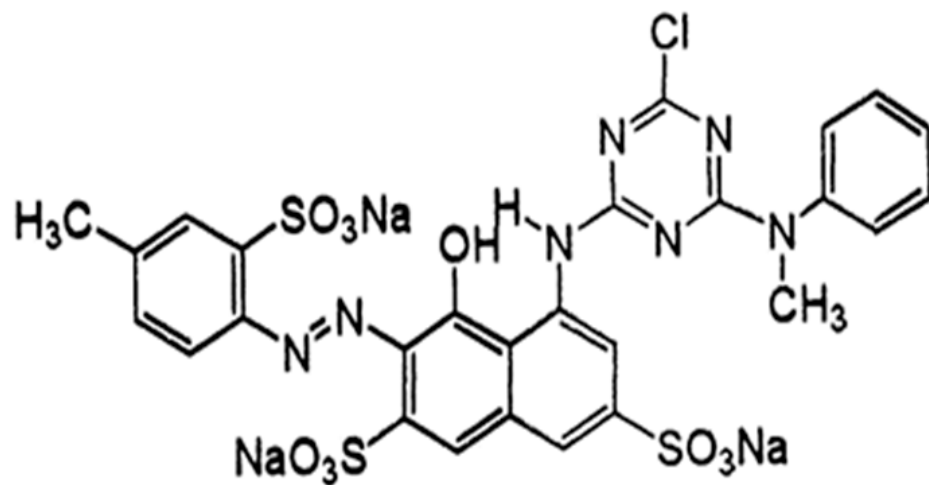
### 2.2. Radiation Treatment of Dye Samples

The dye aqueous solutions (50 mg/L and 100 mg/L) were subjected to UV and UV/H<sub>2</sub>O<sub>2</sub> for 30 min, 60 min, 90 min, 120 min, 150 min, and 180 min of exposure time at the Department of Chemistry, GCUF Pakistan, and also with gamma alone and in conjunction with H<sub>2</sub>O<sub>2</sub> (0.1 mL/L, 0.2 mL/L, 0.3 mL/L, 0.4 mL/L, 0.6 mL/L, 0.8 mL/L,

and 1 mL/L) for the gamma absorbed dose of 0.25 kGy, 0.50 kGy, 0.75 kGy, 1 kGy, 1.50 kGy, and 2 kGy using a Cs-137 gamma radiation source at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The change in absorption at  $\lambda_{\max} = 512$  nm was noted with a UV/Vis Spectrophotometer in the Department of Chemistry, GCUF, Pakistan.

**Table 1.** Characteristics of Reactive Red 45 dye.

Dye Name	Reactive Red 45
Molecular formula	$\text{Na}_3\text{C}_{27}\text{H}_{19}\text{ClN}_7\text{O}_{10}\text{S}_3$
Molecular weight (g/mol)	802.107
Chemical nature	Anionic red 45
Color index name	Red
$\lambda_{\max}$ (nm)	512
Reactive group	Azo group



**Figure 1.** Chemical structure of Reactive Red 45 dye.

### 2.3. Determination of Chemical Oxygen Demand (COD)

The COD change was investigated before and after the irradiation of the samples [21]. The 1 g  $\text{HgSO}_4$  and 50 mL samples were mixed in a flask and then 25 mL of 0.042 M  $\text{K}_2\text{Cr}_2\text{O}_7$  was added after shaking and cooling. A few drops of ferroin indicator were added and titrated against Mohr's salt after refluxing the mixture for 2 h. The color changed from bluish-green to reddish brown and the endpoint was achieved. To get the readings for the blank, the same process was repeated for 50 mL of water as a the blank. The following expression was used for the COD calculations.

$$\text{COD (mg/L)} = \frac{(A - B) * M * 8000}{\text{mL of sample}} \quad (6)$$

Here,

A = FAS volume (mL) for blank

B = FAS volume (mL) for sample

M = FAS concentration (molarity)

### 2.4. Determination of G-Value, Dose Constant (k), $D_{0.50}$ , $D_{0.90}$ , and $D_{0.99}$

The following expression was used to calculate the G-value

$$G = (R) N_A / D (6.4 \times 10^{20}) \quad (7)$$

Here,

R is the change in concentration, D and  $N_A$  are the absorbed dose, and Avogadro's number, respectively. The following expressions were employed to calculate the  $D_{0.50}$ ,  $D_{0.90}$ , and  $D_{0.99}$ .

$$D_{0.50} = \frac{\ln 2}{k} \quad (8)$$

$$D_{0.90} = \frac{\ln 10}{k} \quad (9)$$

$$D_{0.99} = \frac{\ln 100}{k} \quad (10)$$

### 2.5. Toxicity Evaluation

To check the cytotoxicity, 0.80 mg/mL of  $MnO_2$  was added to irradiated solutions to eliminate the effect of residues of hydrogen peroxide before the toxicity evaluation [22]. The solutions after the reaction duration of 1 h were subjected to cytotoxicity evaluation using the reported methods, i.e., *A. cepa* test [23], hemolytic assay [24], brine shrimp (*Artemia salina*) test [25], and Ames test [26].

## 3. Results and Discussion

### 3.1. Effect of Hydrogen Peroxide on the Degradation of Reactive Red 45 Dye

In this study, the Reactive Red 45 dye aqueous solutions were treated with  $H_2O_2$ , and the effect was observed on degradation, pH, and COD before and after treatment. The degradation was noted as 29.29 to 75.33% for 50 mg/L while it was 23.99 to 63.25% for 100 mg/L using 0.1–1 mL/L of oxidant, respectively (Figure 2A). The pH of the solution before adding  $H_2O_2$  was 8.2 which decreased after treatment to 6.8 for 50 mg/L while it was 7.8 for 100 mg/L (Figure 2B). The COD reduction was from 15 to 63% for 50 mg/L, whereas it was from 8 to 52% for 100 mg/L at 0.1–1 mL of concentration of  $H_2O_2$  (Figure 2C).

The increase in the degradation rate and reduction in pH was noted by enhancing the oxidant concentration. At an optimum concentration of  $H_2O_2$ , the benzene ring in the dye was preferentially attacked by the  $\bullet OH$ . When the  $H_2O_2$  concentration exceeded the optimum concentration, probably due to competition between  $H_2O_2$  and the substrate, the  $H_2O_2$  started to scavenge the  $\bullet OH$ -produced perhydroxyl radical ( $\bullet O_2H$ ), which has a lower oxidation capability than  $\bullet OH$  [13]. Our results are consistent with those of Costa et al. [27] whose study on the degradation of textile dyes by  $H_2O_2$  (30%), solar energy, and UV radiation showed 93% degradation. No significant reduction in COD was observed by adding hydrogen peroxide; this indicates that a higher amount of pollutant was present in the water which cannot be reduced by using an oxidant alone. This study suggested that the COD removal was linearly dependent upon the concentration of the oxidant [28].

### 3.2. Effect of Radiation on the Degradation

The dye aqueous solutions (50 mg/L and 100 mg/L) were prepared and treated with UV radiation exposure times of 30 min, 60 min, 90 min, 120 min, 150 min, and 180 min while a gamma radiation absorbed dose of 0.25–2 kGy and degradation efficiency were investigated. The degradation was 33.13%, 44.24%, 48.21%, 56.80%, 64.10%, and 76.02% for 50 mg/L while 27.89%, 40.13%, 45.79%, 51.27%, 57.94%, and 55.78% was observed for 100 mg/L of solution (Figure 3A). Degradation amounts of 41.20%, 59.19%, 67.79%, 70.64%, 80.40%, and 85.54% for 50 mg/L and 31.73%, 52.22%, 60.59%, 62.7%1, 69.96%, and 77.70% for 100 mg/L were obtained (Figure 3B) using UV/ $H_2O_2$ . It was revealed from the results that degradation increased as the exposure time of UV radiation enhanced, and a maximum degradation (85.54%) was obtained at 180 min. After that, the intermediate species were produced and a decrease in the degradation was observed due to the competition between intermediates.

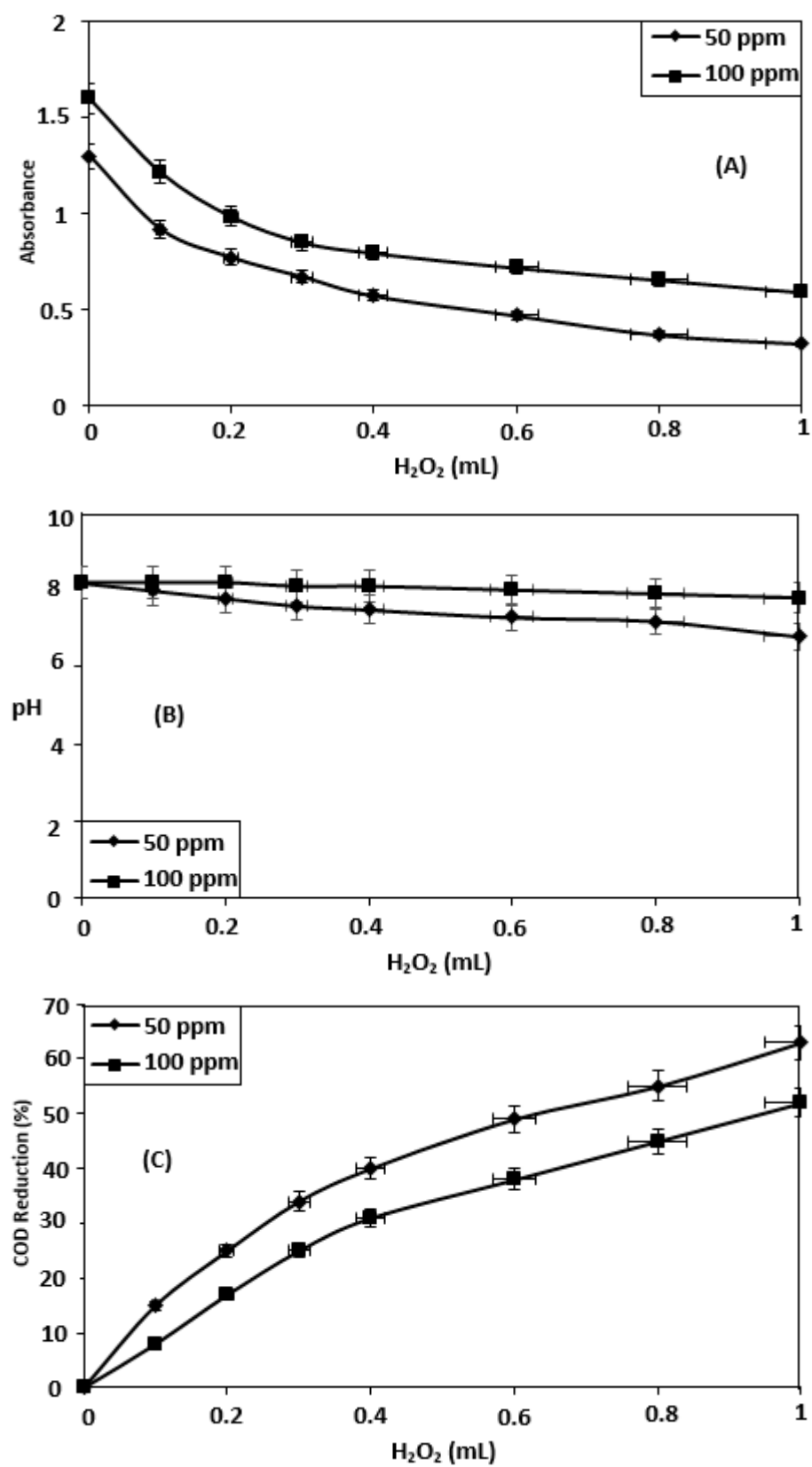
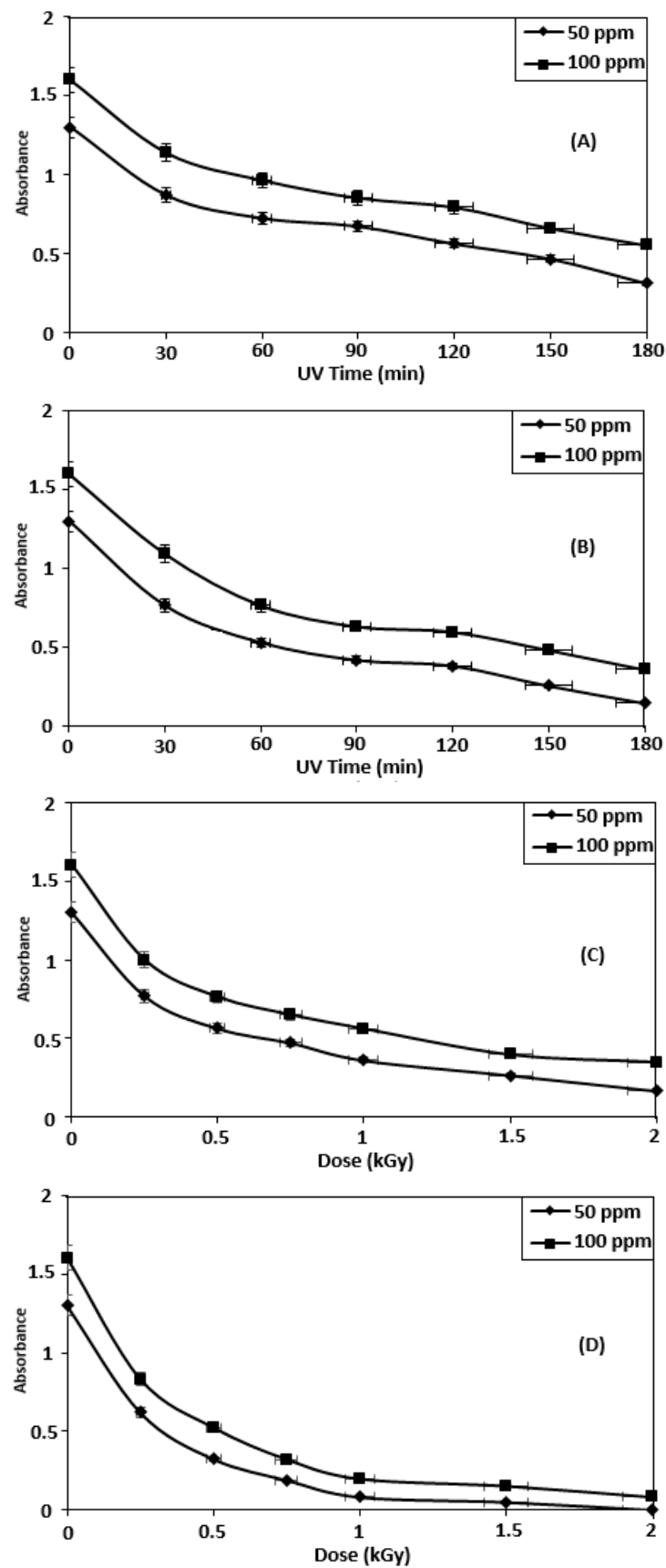


Figure 2. Effect of hydrogen peroxide on Reactive Red 45 dye aqueous solutions: (A) Absorbance, (B) pH, and (C) COD.



**Figure 3.** Effect of radiation on the absorbance of Reactive Red 45 dye aqueous solutions: (A) UV, (B) UV/H<sub>2</sub>O<sub>2</sub>, (C) Gamma, and (D) Gamma/H<sub>2</sub>O<sub>2</sub>.

The degradation was enhanced to 40.81%, 56.57%, 63.64%, 72.18%, 79.48%, and 89.09% for 50 mg/L while it was 37.60%, 52.22%, 59.28%, 64.83%, 75.08%, and 78.25% for 100 mg/L by treating with gamma radiation (Figure 3C). Whereas, 52.27%, 75.02%, 85.32%, 93.62%, 96.08%, and 100% for 50 mg/L and 48.03%, 67.27%, 79.83%, 87.57%, 90.44%, and 94.82% degradation for 100 mg/L (Figure 3D) were achieved by using gamma/H<sub>2</sub>O<sub>2</sub> at a gamma absorbed dose of 2 kGy.

The obtained results showed improvement in the degradation when the solutions were subjected to gamma radiation in conjunction with H<sub>2</sub>O<sub>2</sub>. The optimum dose of H<sub>2</sub>O<sub>2</sub> is recommended to obtain better results as the H<sub>2</sub>O<sub>2</sub> acts as a promotor and radical scavenger. It was concluded that the ultimate effect of UV, as well as gamma radiation on the dye solution, is the removal of the chromophore group. Similar indications were observed by Ma et al. [29] who treated the wastewater using AOP to degrade the organic matter to purify the water.

### 3.3. Effect of Radiation on Chemical Oxygen Demand (COD)

The dye solutions were treated with UV and gamma radiation and COD evaluation was carried out before and after each radiation treatment. The reduction in COD was 22%, 33%, 45%, 54%, 60%, and 66% for 50 mg/L and 16%, 25%, 36%, 44%, 53%, and 58% for 100 mg/L observed by applying UV radiation for 30–180 min (Figure 4A). The removal in COD was increased to 28%, 42%, 53%, 62%, 70%, and 78% for 50 mg/L and 20%, 32%, 41%, 49%, 58%, and 64% for 100 mg/L by using UV/H<sub>2</sub>O<sub>2</sub> (Figure 4B). It was inferred that the COD reduced significantly (78%) after UV/H<sub>2</sub>O<sub>2</sub> treatment which is due to the breakdown of larger organic molecules into simple and less toxic compounds. An improvement in COD removal was obtained by treating the samples with gamma radiation. The COD was reduced to 25–70% for 50 mg/L and 17–64% for 100 mg/L at a 0.25–2 kGy gamma absorbed dose (Figure 4C). The COD reduction was enhanced by treating the samples with gamma/H<sub>2</sub>O<sub>2</sub> which was 31–85% for 50 mg/L and 25–77% for 100 mg/L (Figure 4D). The rate of COD removal was prominent in the presence of H<sub>2</sub>O<sub>2</sub> because it produced more hydroxyl radicals which ultimately increased the rate of the oxidation of dye.

### 3.4. Effect of Radiation on pH of Reactive Red 45 Dye Aqueous Solutions

The dye aqueous solutions were treated with UV and gamma radiation alone and in conjunction with H<sub>2</sub>O<sub>2</sub> and the change in pH was determined for untreated and treated samples. The pH of the untreated sample was 8.2 which reduced to 7.8, 7.5, 7.3, 7.1, 6.9, and 6.7 for 50 mg/L and 8, 7.9, 7.7, 7.5, 7.3, and 7.1 for 100 mg/L at 30–180 min of UV exposure time (Figure 5A). The pH was further reduced to 7.6, 7.4, 4.3, 7.1, 6.8, and 6.5 for 50 mg/L and 7.9, 7.6, 7.5, 7.3, 7.1, and 6.8 for 100 mg/L after UV/H<sub>2</sub>O<sub>2</sub> treatment (Figure 5B). The pH decreased after gamma radiation and the pH reduced to 7.6, 7.4, 7.1, 6.8, 6.6, and 6.3 for 50 mg/L and 7.8, 7.6, 7.3, 7.1, 6.8, and 6.5 for 100 mg/L (Figure 5C). The effect was more severe for gamma/H<sub>2</sub>O<sub>2</sub> and the pH decreased to 7.3, 7.1, 6.8, 6.5, 6.2, and 5.7 for 50 mg/L and 7.5, 7.3, 7.1, 6.7, 6.4, and 6.1 for 100 mg/L after gamma/H<sub>2</sub>O<sub>2</sub> treatment (Figure 5D).

There was negligible change in pH by using UV and H<sub>2</sub>O<sub>2</sub> alone which indicated that the UV and H<sub>2</sub>O<sub>2</sub> alone are not capable of degrading the sample but reduced the pH when using the UV/H<sub>2</sub>O<sub>2</sub> process. The reduction in pH was more prominent using gamma/H<sub>2</sub>O<sub>2</sub> which might be due to the breakdown of complex molecules into smaller molecules of low molecular weight aliphatic acids such as formic acid, maleic acid, etc., shifting the pH to the lower side. Similar results were reported by a number of authors [3,30,31] showing that the structure of the chromophore group was decomposed into small acidic compounds such as aliphatic carboxylic acid during the degradation of the dye solution to lower the pH.

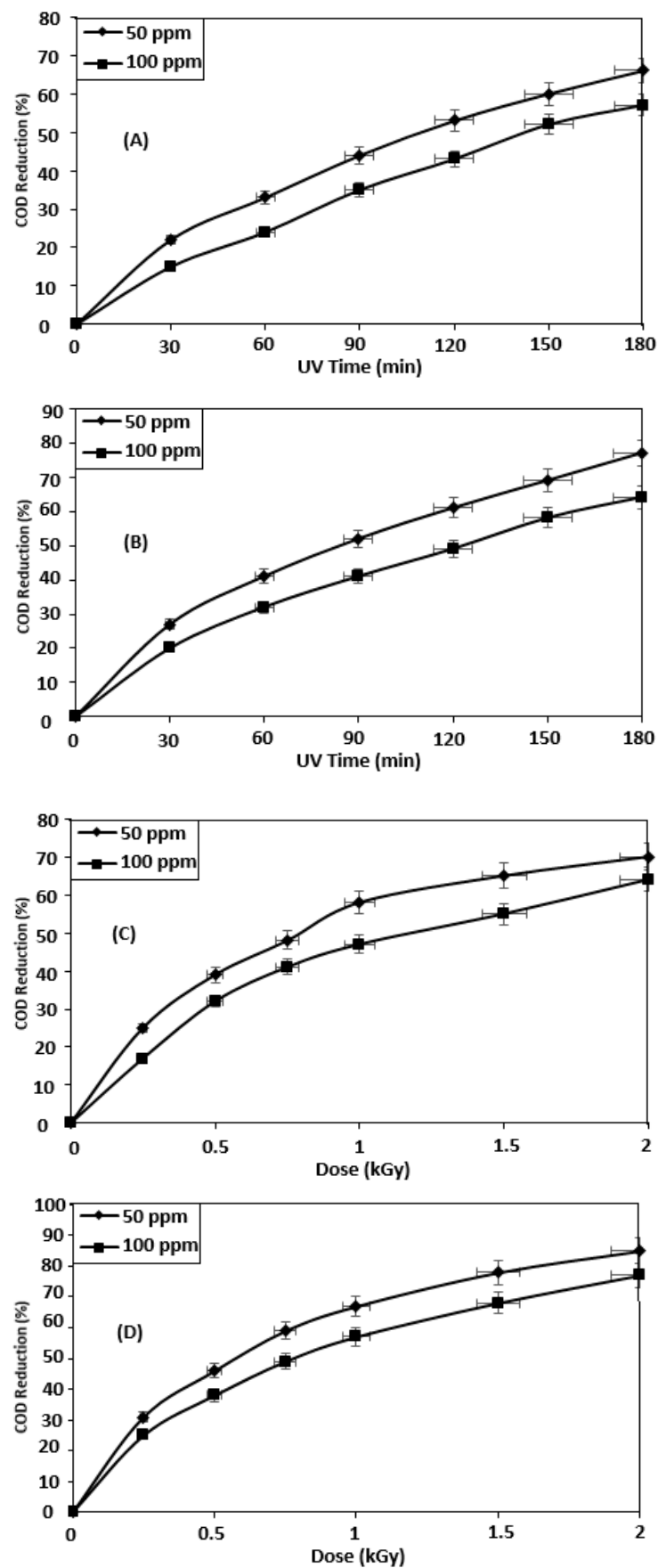


Figure 4. Effect of radiation on the COD of Reactive Red 45 dye aqueous solutions: (A) UV, (B) UV/H<sub>2</sub>O<sub>2</sub>, (C) Gamma, and (D) Gamma/H<sub>2</sub>O<sub>2</sub>.

### 3.5. Dose Constant ( $k$ ), Removal Efficiency ( $G$ -Value), $D_{0.50}$ , $D_{0.90}$ , and $D_{0.99}$

Table 2 shows the observed data of the  $G$ -value,  $k$ ,  $D_{0.50}$ ,  $D_{0.90}$ , and  $D_{0.99}$  of the gamma-ray-treated samples along with  $H_2O_2$ . The results indicated a reduction in  $G$ -value but significant improvement in  $D_{0.50}$ ,  $D_{0.90}$ , and  $D_{0.99}$  by increasing the dye initial concentration. The results suggest that an elevated absorbed dose of gamma radiation is required to remove 50%, 90%, and 99% dye initial concentration to overcome the competition between intermediates and parent compounds [28]. At a constant dye initial concentration, a significant improvement in  $G$ -value was observed for gamma-irradiated samples along with  $H_2O_2$ .

**Table 2.** Removal efficiency, dose constant,  $D_{0.50}$ ,  $D_{0.90}$ , and  $D_{0.99}$  for degradation of Reactive Red 45 dye aqueous solutions.

Treatment Dye	Absorbed Dose		G-Value	Dose Constant	$D_{0.50}$	$D_{0.90}$	$D_{0.99}$
	(ppm)	(kGy)	( $\mu\text{mol/l}$ )	( $\mu\text{M/kGy}$ )	(kGy)	(kGy)	(kGy)
Gamma	50	0.25–2	0.9814–0.0228	0.944	0.734	2.439	4.878
	100	0.25–2	1.8098–0.0181	0.714	0.969	3.221	6.443
Gamma/ $H_2O_2$	50	0.25–2	1.2577–0.0117	2.028	0.034	1.135	2.270
	100	0.02–2	2.3119–0.0263	1.415	0.489	1.626	3.252

### 3.6. Cytotoxicity of Reactive Red 45 Dye

During the treatment of dye aqueous solutions, more toxic compounds may be generated which might be dangerous for aquatic life. Therefore, careful treatment of the effluent is a major concern [32–35]. The bioassays such as hemolytic, brine shrimp (*Artemia salina*), and *A. cepa* tests were carried out for the cytotoxicity evaluation before and after UV and gamma radiation, and the results obtained in the case of the *A. cepa* tests are mentioned in Tables 3 and 4.

**Table 3.** *Allium cepa* test exhibiting cytotoxicity reduction after UV-radiation treatment.

Sample	Exposure Time (min)	<i>A. cepa</i> Test					
		Root Length (cm)		Root Count		Mitotic Index	
Untreated		4.2 $\pm$ 0.52		14 $\pm$ 0.63		12 $\pm$ 0.28	
Positive control		9.3 $\pm$ 0.77		29 $\pm$ 0.45		32 $\pm$ 0.74	
Negative control		3.5 $\pm$ 0.63		11 $\pm$ 0.23		14 $\pm$ 0.66	
		UV alone	UV + $H_2O_2$	UV alone	UV + $H_2O_2$	UV alone	UV + $H_2O_2$
Sample 1	30	4.9 $\pm$ 0.68	5.4 $\pm$ 0.44	15 $\pm$ 0.44	17 $\pm$ 0.68	15 $\pm$ 0.47	18 $\pm$ 0.69
Sample 2	90	5.5 $\pm$ 0.31	6.2 $\pm$ 0.65	18 $\pm$ 0.31	20 $\pm$ 0.74	19 $\pm$ 0.31	21 $\pm$ 0.74
Sample 3	180	6.3 $\pm$ 0.67	6.9 $\pm$ 0.57	20 $\pm$ 0.44	22 $\pm$ 0.76	22 $\pm$ 0.44	23 $\pm$ 0.13

**Table 4.** *Allium cepa* test exhibiting cytotoxicity reduction after gamma radiation treatment.

Sample	Absorbed Dose (kGy)	<i>A. cepa</i> Test					
		Root Length (cm)		Root Count		Mitotic Index	
Untreated		4.2 $\pm$ 0.52		14 $\pm$ 0.63		12 $\pm$ 0.28	
Positive control		9.3 $\pm$ 0.77		29 $\pm$ 0.45		32 $\pm$ 0.74	
Negative control		3.5 $\pm$ 0.63		11 $\pm$ 0.23		14 $\pm$ 0.66	
		Gamma alone	Gamma + $H_2O_2$	Gamma alone	Gamma + $H_2O_2$	Gamma alone	Gamma + $H_2O_2$
Sample 1	0.25	5.9 $\pm$ 0.62	6.5 $\pm$ 0.44	19 $\pm$ 0.42	21 $\pm$ 0.61	20 $\pm$ 0.41	21 $\pm$ 0.62
Sample 2	0.75	6.7 $\pm$ 0.31	7.4 $\pm$ 0.65	21 $\pm$ 0.51	25 $\pm$ 0.24	23 $\pm$ 0.61	24 $\pm$ 0.44
Sample 3	2	7.8 $\pm$ 0.65	8.2 $\pm$ 0.55	24 $\pm$ 0.43	27 $\pm$ 0.73	25 $\pm$ 0.42	28 $\pm$ 0.12

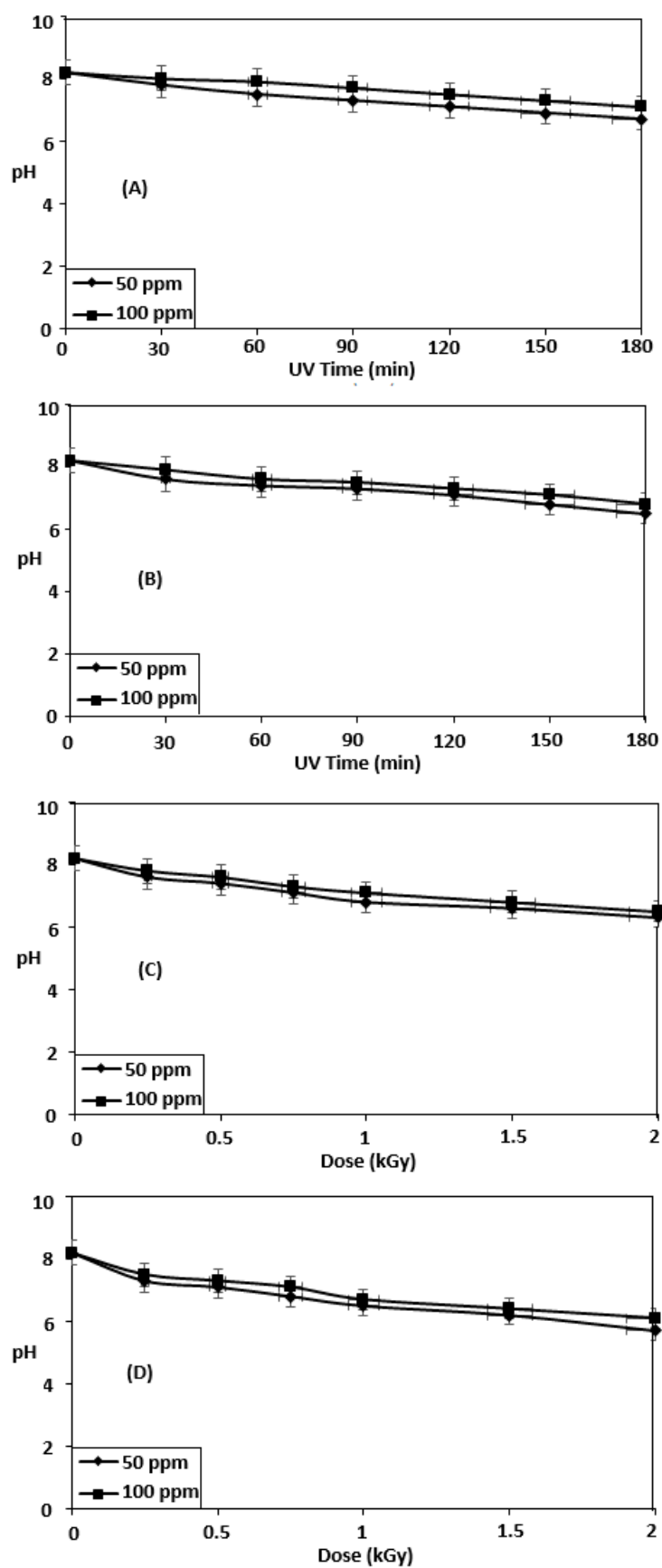


Figure 5. Effect of radiation on the pH of Reactive Red 45 dye aqueous solutions: (A) UV, (B) UV/H<sub>2</sub>O<sub>2</sub>, (C) Gamma, and (D) Gamma/H<sub>2</sub>O<sub>2</sub>.

Before treatment, RC, RL, and MI values were measured to be 14, 4.2 cm, and 12, respectively, while the observed increase was up to 36%, 39.11%, and 47.82% in the *A. cepa* roots using UV/H<sub>2</sub>O<sub>2</sub>, and a clear increment of up to 42%, 46.36%, and 56% in RC, RL, and MI was noted in the case of gamma/H<sub>2</sub>O<sub>2</sub>. Our results correlate with the findings of Jadhav et al. [35] who determined that the mitotic index before the radiation treatment was 11.68% which was increased up to 14.25% after the radiation treatment. Recently, Iqbal and Nisar [31] treated the textile effluent photo-catalytically and found a 41.17% and 41.12% increase in root count and root length, respectively, in the *A. cepa* test [31]. The cytotoxicity of Reactive Red 45 dye can also be checked by using the brine shrimp (*Artemia salina*) test [31]. This test is based on the killing ability of nauplii cultured in the laboratory at a given time. Before the radiation treatment, the death rate of cultured brine shrimp nauplii was 88% when exposed to dye solution which was decreased to 21% and 16% when using UV/H<sub>2</sub>O<sub>2</sub> and gamma/H<sub>2</sub>O<sub>2</sub> processes, respectively (Tables 5 and 6).

**Table 5.** Brine shrimp (*Artemia salina*) test exhibiting the reduction in cytotoxicity after UV radiation treatment.

Sample	Exposure Time (min)	Brine Shrimp Test	
		% Age Death (after 24 h)	
Untreated		88 ± 0.27	
Positive control		100 ± 0.0	
Negative control		0	
		UV alone	UV + H <sub>2</sub> O <sub>2</sub>
Sample 1	30	26 ± 0.67	20 ± 0.85
Sample 2	90	22 ± 0.53	16 ± 0.35
Sample 3	180	19 ± 0.81	14 ± 1.04

Guelli Souza et al. [36] determined that the sample treated with peroxidase enzyme showed a significant reduction (four times) in toxicity than untreated textile effluent using bioassays. The hemolytic activity of RBCs was carried out for the untreated and treated samples by UV/H<sub>2</sub>O<sub>2</sub> and gamma/H<sub>2</sub>O<sub>2</sub> for toxicity assessment (Tables 7 and 8).

**Table 6.** Brine shrimp (*Artemia salina*) test exhibiting the reduction in cytotoxicity after gamma radiation treatment.

Sample	Absorbed Dose (kGy)	Brine Shrimp ( <i>Artemia salina</i> ) Test	
		% Age Death (after 24 h)	
Untreated		88 ± 0.27	
Positive control		100 ± 0.0	
Negative control		0	
		Gamma alone	Gamma + H <sub>2</sub> O <sub>2</sub>
Sample 1	0.25	23 ± 0.68	17 ± 0.85
Sample 2	0.75	28 ± 0.63	12 ± 0.35
Sample 3	2	25 ± 0.82	8 ± 1.01

**Table 7.** Hemolytic test exhibiting cytotoxicity reduction after UV radiation treatment.

Sample	Exposure Time (min)	Hemolytic Test (% Age Hemolysis)	
		Positive control	96.23 ± 0.82
Negative control	2.11 ± 0.32	2.11 ± 0.32	
		UV alone	UV + H <sub>2</sub> O <sub>2</sub>
Sample 1	30	16.32 ± 0.55	18.52 ± 0.28
Sample 2	120	17.56 ± 0.75	19.36 ± 0.76
Sample 3	180	19.23 ± 0.56	21.25 ± 0.53

**Table 8.** Hemolytic test exhibiting cytotoxicity reduction after gamma radiation treatment.

Sample	Absorbed Dose (kGy)	Hemolytic Test (% Age Hemolysis)	
		Gamma alone	Gamma + H <sub>2</sub> O <sub>2</sub>
Positive control		96.23 ± 0.82	96.23 ± 0.82
Negative control		2.11 ± 0.32	2.11 ± 0.32
Sample 1	0.25	17.57 ± 0.28	19.56 ± 0.58
Sample 2	0.75	18.62 ± 0.66	21.34 ± 0.37
Sample 3	2	20.25 ± 0.51	23.21 ± 0.69

The obtained data showed a clear reduction in toxicity after UV/H<sub>2</sub>O<sub>2</sub> and gamma/H<sub>2</sub>O<sub>2</sub> treatments. However, a greater reduction was observed in the case of gamma/H<sub>2</sub>O<sub>2</sub>. Up to 21.25% and 23.21% hemolysis were observed after UV/H<sub>2</sub>O<sub>2</sub> and gamma/H<sub>2</sub>O<sub>2</sub> treatments, respectively. The dye aqueous solutions were found to be toxic to *A. cepa* root tip cells, RBCs, and brine shrimp (*Artemia salina*) nauplii reduced significantly after UV/H<sub>2</sub>O<sub>2</sub> and gamma/H<sub>2</sub>O<sub>2</sub> treatments. However, the samples treated with gamma/H<sub>2</sub>O<sub>2</sub> showed a considerable reduction in toxicity compared with UV/H<sub>2</sub>O<sub>2</sub>. The obtained results reveal that gamma/H<sub>2</sub>O<sub>2</sub> is more suitable than UV/H<sub>2</sub>O<sub>2</sub> for the degradation and detoxification of dye effluents. Recently, Iqbal and Nisar [31] treated the textile effluent by using gamma radiation along with H<sub>2</sub>O<sub>2</sub> and found 42.12% hemolysis in the hemolytic test.

### 3.7. Mutagenicity Evaluation of Reactive Red 45 Dye

The Ames test is a validated and reference test that can be used for mutagenic evaluation [37]. Tables 9 and 10 show the mutagenic evaluation for untreated and treated dye solutions using TA98 and TA100 bacterial strains.

**Table 9.** The Ames test exhibiting mutagenicity reduction after UV radiation treatment.

Sample	Exposure Time (min)	AMES Test (% Age Mutagenicity)			
		TA98 Count		TA100 Count	
-					
Standard Background		77.08 ± 0.46		70.83 ± 0.91	
		33 ± 0.71		29.17 ± 0.85	
-		UV alone	UV + H <sub>2</sub> O <sub>2</sub>	UV alone	UV + H <sub>2</sub> O <sub>2</sub>
Blank	-	39.58 ± 0.33	31.08 ± 0.65	35.42 ± 0.55	32.24 ± 0.41
Sample 1	30	31.25 ± 0.19	28.13 ± 0.48	33.33 ± 0.42	29.12 ± 0.25
Sample 2	120	27.08 ± 0.43	16.67 ± 0.47	28.13 ± 0.18	19.79 ± 0.81
Sample 3	180	20.83 ± 0.45	12.5 ± 0.58	21.88 ± 0.66	15.67 ± 0.76

**Table 10.** The Ames test exhibited mutagenicity reduction after gamma radiation treatment.

Sample	Radiation Dose (kGy)	AMES Test (% Age Mutagenicity)			
		TA98 Count		TA100 Count	
-					
Standard Background		77.08 ± 0.46		70.83 ± 0.91	
		33 ± 0.71		29.17 ± 0.85	
-		Gamma alone	Gamma + H <sub>2</sub> O <sub>2</sub>	Gamma alone	Gamma + H <sub>2</sub> O <sub>2</sub>
Blank	-	39.58 ± 0.35	31.08 ± 0.82	35.42 ± 0.29	32.24 ± 0.41
Sample 1	0.25	29.17 ± 0.25	19.79 ± 0.51	30.24 ± 0.25	23.96 ± 0.35
Sample 2	0.75	23.96 ± 0.91	13.54 ± 0.26	25.32 ± 0.79	18.75 ± 0.48
Sample 3	2	17.71 ± 0.34	9.38 ± 0.85	20.83 ± 0.34	13.54 ± 0.37

After UV and gamma radiation treatment, the mutagenicity reduced significantly. The non-mutagenic behavior against TA98 and TA100 strains was observed in the solutions which were treated with UV and UV/H<sub>2</sub>O<sub>2</sub> (31.25%, 27.08%, and 20.83% for TA98, and 33.33%, 28.13%, and 21.88% for TA100 with UV radiation alone while 28.13%, 16.67% and 12.5% for TA98 and 29.12%, 19.79%, and 15.67% along with H<sub>2</sub>O<sub>2</sub> at 30, 120 and 180-min)

respectively. The mutagenicity was decreased for the gamma-irradiated samples along with oxidant towards TA98 as well as the TA100 strain (29.17%, 23.96%, and 17.71% for TA98, while 30.24%, 25.32%, and 20.83% for TA100 with gamma radiation alone, and 19.79%, 13.54%, and 9.38% for TA98 while 23.96%, 18.75%, and 13.54% with H<sub>2</sub>O<sub>2</sub> at 0.25, 0.75 and 2 kGy absorbed dose, respectively). The obtained data revealed that the treated solutions with UV and gamma radiation along with H<sub>2</sub>O<sub>2</sub> showed more non-mutagenic behavior towards TA98 and TA100 strains and can be released into the environment without causing harm [31]. This is due to the metabolized reaction in the living cells to convert them into chemical products to react with cellular macromolecules for participation in redox reactions [38,39].

#### 4. Conclusions

It was concluded from the results of this study that the toxic compounds present in the textile effluent can efficiently be degraded using gamma radiation along with H<sub>2</sub>O<sub>2</sub> compared with UV radiation. The toxicity of the treated samples was reduced significantly. A high dose of gamma radiation or electron beam accelerator can treat the wastewater in a very low time frame without storage of wastewater. The *A. cepa*, brine shrimp (*Artemia, salina*), and hemolytic tests proved to be better tools for the measurement of toxicity, and mutagenicity was efficiently evaluated using the Ames test. Furthermore, it was observed that dye aqueous solutions were cytotoxic and mutagenic; however, these can efficiently be detoxified by the degradation using advanced oxidation processes (Gamma/H<sub>2</sub>O<sub>2</sub> and UV/H<sub>2</sub>O<sub>2</sub>), and these treatment methods might be an interesting alternative, especially for the degradation of effluents contaminated with cytotoxic and mutagenic agents.

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