## Degradation of Cytotoxic Agent in Soap and Detergent Wastewater by Advanced Oxidation Processes

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**Summary:** Wastewater from soap and detergent industries is a source of high pollution and contamination for water sheds. In present investigation, cytotoxic profiling was documented from Faisalabad, Sargodha and Gujranwala cities, Pakistan, followed by advanced oxidation processes (AOPs) treatments (UV and gamma radiation). The cytotoxicity was evaluated by *Allium cepa*, haemolytic and brine shrimp bioassays. Independent variables such as gamma radiation absorbed dose,  $H_2O_2$ , TiO<sub>2</sub> concentrations, reaction time, pH and shaking speed were optimized using statistical techniques. The raw soap and detergent wastewater showed cytotoxicity up to high extent. At optimized conditions, > 94% degradation was achieved both in case of UV (exposure time 100 min, TiO<sub>2</sub> concentration 5.93 g/L,  $H_2O_2$  4.39%, pH 6.50 and shaking speed 110 rpm) and gamma radiation (12.69 kGy absorbed dose in the presence of 4.65%  $H_2O_2$ ) treated samples and water quality parameters (WQP) also improved significantly. The cytotoxicity reduced sharply as a result of AOPs treatment at optimized conditions. From the results, it is evident that AOPs under investigation could be used for the degradation and cytotoxicity reduction of soap and detergent wastewater.

**Keywords:** Soap and detergents wastewater; Advanced oxidation; Absorbed dose, Cytotoxicity; Bioassays; Water quality parameters.

#### Introduction

Synthetic soaps and detergents are used by every industry as essential raw materials. Moreover, they are also being used frequently in our daily life in everything from hair shampoo and clothes washing powder to shaving foam and stain removers. However, despite their increased usage, relatively little work has been focused on their environmental effects. These effluents contain endocrine disruptors (surfactants) and due to their weak ability to mimic estrogen, they in turn disrupt the natural balance of hormones in exposed organisms [1, 2]. The soaps and detergents wastewater have many of the common organic pollutants and enter in to the environment via wastewater discharges as a result of their widespread use in aqueous solutions. After use, consumer detergents are usually discarded down the drain into municipal sewer systems and resultantly, mixed with water sheds [3]. The chemicals used in toilet products, fabric washing, dish cleaning, surface cleaning and shampoos are rather simple since the products are subject to wide dispersive use with "down-the-drain" disposal. Overall, most of the losses to the environment occur at the use phase and for the purposes of exposure modeling it is assumed that 100% emission to sewer occurs at the post consumer stage. The annual production of detergents in the USA, Western Europe and Japan is  $6 \times 10^6$  tones. The wastewater from the manufacturing of detergents, which contains residual product material, can have a very variable pollution load and if left untreated, can have an appreciable impact on the environment [4, 5].

AOPs have been employed successfully for the treatment of industrial wastewater and studies regarding effects on toxicity reduction are also undertaken in last decade (Table-1) [6]. During AOPs treatment strong oxidizing species like OH' are produced in situ, which break down the complex organic molecule into harmless end products like CO<sub>2</sub>, H<sub>2</sub>O and inorganic ions through a chain reactions [7-9]. These processes are easy to handle, produce significantly less residual effects as compared to classical treatment approaches and are being employed for color removal and mineralization of toxic chemicals from industrial wastewater [10-12]. Currently, the trend in the development of new innovative technologies is focused on those processes that eventually cause destruction of the organic contaminants rather than transferring them into another form. The application of high energy radiation has been accepted efficient in this regard [13-15]. The radiation induced degradation of wastewater by gamma radiation, electron beam and UV radiation is drawing more attention around the world. The treatment of wastewater effluents by radiation has many advantages such as it degrades the non biodegradable organic system, does not cause secondary pollution and is cost effective and eco-friendly [16-18].

To the best of our knowledge, the cytotoxicity of soap and detergent wastewater has not been reported using *allium cepa*, haemolytic and brine shrimp bioassays and there is also lack of information whether UV and gamma radiation are efficient to degrade the pollutant in soap and detergent wastewater. Therefore, the present study was performed to explore the possibility of UV and gamma radiation for the degradation and cytotoxicity reduction of soap and detergent wastewater. The UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> and gamma radiation/H<sub>2</sub>O<sub>2</sub> were used and efficiencies were evaluated on the basis of degradation, water quality parameter (WQP) and cytotoxicity reduction.

### Experimental

#### Chemical, reagents and instruments

Hydrogen peroxide (35%), titanium dioxide (p25 degussa), methyl methanesulfonate (99%),

Cyclophosphamide (CAS Number 6055-19-2), Triton X-100 (CAS Number 9002-93-1), acetone ( $\geq$ 99.5%), ethanol ( $\geq$ 99.8%), NaCl ( $\geq$ 99.5%), KH<sub>2</sub>PO<sub>4</sub> ( $\geq$ 99%), Na<sub>2</sub>HPO<sub>4</sub> ( $\geq$ 99%), KCl ( $\geq$ 99%), NaOH ( $\geq$ 98%) and HCl (37%) were purchased from Sigma-Aldrich. Digital pH meter (Lovibond Senso Direct 150, Germany), COD meter (Lovibond OxDirect, Germany), UV/Vis double beam spectrophotometer (CE Cecil 7200, Germany) were used for analysis throughout the study.

#### Sample Collection

For sample collection, three soap and detergent units were selected from the industrial cities Faisalabad, Sargodha and Gujranwala, Pakistan using standard sampling methods [12]. Briefly, the plastic gallon were pre-cleaned by soaking in nitric acid 1% (v/v) for 24 h and rinsed with distilled water. Triplicate samples were collected from each industry. The collected samples were transported to respective laboratories, Radiation Chemistry Laboratory and Protein & Molecular laboratory (PML), Bioassay Section, University of Agriculture, Faisalabad, Pakistan and stored at 4°C.

Table-1: Rep	ported bioassay	used in evaluating	toxicity	of wastewater/simul	lated solutions the	reated by AOPs.
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Bioassay	AOPs investigated	Tested system	
V. fischeri	O3 (1 mmol L <sup>-1</sup> , 0.38 L min <sup>-1</sup> flowrate)	Bezafibrate (0.2– 0.5 mmol L <sup>-1</sup> )	
Ankistrodesmus braunii and S. capricornutum	$O_3$ (2.0% per volume, 36 dm <sup>3</sup> h <sup>-1</sup> flowrate).	Carbamazepine (3.3 × 10 <sup>-6</sup> mmol dm <sup>-3</sup> )	
D. magna, P. subcapitata and A. salina	$TiO_2/UV (0.2-1.6 \text{ g } \text{L}^{-1})$	Diclofenac (5–80 mg L <sup>-1</sup> )	
P. subcapitata,Cyclotella meneghiniana,Synechococcus leopoliensis.	Sunlight irradiation, UV/H <sub>2</sub> O <sub>2</sub> (254 nm low-pressure lamp) and O <sub>3</sub> .	Lyncomicin (0.5 mM)	
S. leopoliensis,Brachyonus calyciflorus	O <sub>3</sub> (0.42 mM, 36 dm <sup>3</sup> h <sup>-1</sup> flowrate), H <sub>2</sub> O <sub>2</sub> /UV (5 and 10 mM of H <sub>2</sub> O <sub>2</sub> ) and TiO <sub>2</sub> /UV (suspended or immobilized TiO <sub>2</sub> , 0.3 g L <sup>-1</sup> , Degussa, 300 W sunlight simulator, up to 48 h contact time)	Mixture of six pharmaceuticals	
P. putida	UV/TiO <sub>2</sub> , electro-Fenton, wet-air oxidation, and UV/electro-Fenton.	Reactive Red 120 (20– 100 mg L <sup>-1</sup> )	
V. fischeri, D. magna and S. capricornotum	Solar driven photo-Fenton and ${\rm TiO_2}$ photocatalysis pilot plant	Methomyl (50 mg $L^{-1}$ )	
D. magna andBacillus subtilis sp.	Photo-Fenton (2 L reactor, three 6 W Philips black-light fluorescent lamps $(I = 5 \times 10^{-6} \text{ Einstein s}^{-1})$ , controlled temperature (25 °C)	Imidacloprid (100 mg $L^{-1}$ )	
D. magna,Photobacterium phosphoreum, umu (genotoxicity) test	$O_3$ and $O_3/UV$ (40 mg $L^{-1}$ $O_3 dosage,$ 20 and 40 min treatment), $H_2O_2$ and $H_2O_2/UV$ (6 mL $L^{-1}$ of 30% $H_2O_2$ )	Mixture of municipal and industrial wastewater pre- treated by MBR	
V. fischeri (toxicity) and <i>Salmonella</i> typhimurium(mutagenicity)	Ozonation (applied ozone 2.5–8.0 mg $L^{-1}$ , 2–30 min treatment)	Effluent from secondary biological treatment	
D. magna, P. subcapitata, L. sativum	TiO <sub>2</sub> photocatalysis, catalyst loading in the range of 0.2–0.8 g L <sup>-1</sup> , 125 W black- light fluorescent lamp, 120 min maximum irradiation time.	Effluent from secondary biological treatment	
FELST with rainbow trout (Oncorhynchus mykiss)	Ozonation (maximum applied ozone concentration 1 mg O <sub>3</sub> /mg DOC)	Effluent from secondary biological treatment plant	
D. longispina.	Photo-Fenton process before and after biological treatment by three species of fungi	Diluted and undiluted wastewater samples from olive mill plant	
P. subcapitata and phytotoxicity to seeds of R. sativus, C. sativus and L. sativa	Ozonation, solar photolysis, solar modified photo-Fenton, solar modified photo-Fenton–ozonation.	Centrifuged wastewater sample	
A. salina	H <sub>2</sub> O <sub>2</sub> /UV, TiO <sub>2</sub> /H <sub>2</sub> O <sub>2</sub> /UV and TiO <sub>2</sub> /UV in a continuous operated annular reactor (15 W UV lamp 1 g TiO <sub>2</sub> $L^{-1}$ 1 h)	Coagulated/settled	

### Treatment Procedure

For UV irradiation, the UV radiation source (55 W, mercury lamp, wavelength 354 nm, Galvano, Pakistan) was used. The soap and detergent wastewater was filtered before irradiation and subjected to UV irradiation and independent variables H<sub>2</sub>O<sub>2</sub> (1.5-5.5%) and TiO<sub>2</sub> (3-7 g/L), pH (6-12), UV exposure time (40-120 min) and shaking speed (50-150 rpm) under RCCD were investigated. After TiO<sub>2</sub> addition, the mixture was stirred for 30 min without irradiation in order to get adsorptiondesorption equilibrium. After stipulated irradiation period, the samples were centrifuged to separate the catalyst. Absorbance was monitored at 274 nm (CE Cecil 7200, Germany) and degradation was calculated using Eq. 1. Where D is percentage degradation,  $A_i$  and  $A_f$  are OD's before and after irradiation, respectively.

$$D(\%) = \frac{A_i - A_f}{A_i} \times 100$$
 (Eq. 1)

For gamma irradiation, Cs-137 gammaradiation source at Nuclear Institute of Agriculture and Biology (NIAB) was used. The samples were irradiated to the absorbed doses of 5, 10 and 15 kGy in the presence of 1.5%, 3.5% and 5.5% H<sub>2</sub>O<sub>2</sub> for each dose. Un-irradiated sample were used as control under similar conditions except irradiation. The dose rate at the time of sample irradiation was 1.25 kGy  $h^{-1}$  and radiation source was calibrated by Fricke dosimeter and dose rate was calculated using Eq. 2.

$$D = \left\{ \frac{N \times \Delta OD \times 100}{c \times p \times G(Fe3+)} \right\} ev/g \qquad (Eq. 2)$$

where, D is dose, OD is the difference in absorbance of irradiated and un-irradiated solutions at 304 nm, N  $10^{23}$ number (6.023 is the Avogadro x molecule/mole),  $\epsilon$  is the molar extinction co-efficient of the ferric ions (2205 M<sup>-1</sup>cm<sup>-1</sup> at 304 nm at room temperature),  $\rho$  is the density of dosimetric solution  $(1.024 \text{ g/cm}^3 \text{ for } 0.4 \text{ M H}_2\text{SO}_4)$  and G (Fe<sup>+3</sup>) is the radiation yield (number of ions formed per 100 ev of absorbed energy (15.6 for Fricke solution)) (Sehested, 1970). To avoid uncertainty, transient dose (Eq. 3) was measured and was added to "D" for accurate absorbed dose measurement. The absorbed dose for irradiated sample was calculated by adding D and D (T) from Eqs. 2 and 3 as shown in Eq. 4, where D (T) is transient dose and "t" is time. The percentage degradation of gamma irradiated sample was measured using Eq. 1.

# $D(T) = \{D \ge \Delta OD \text{ at zero } t\}$ Rads Eq. 3

# Absorbed dose = $D \times D(T) \text{ kGy h}^{-1}$ Eq. 4

## Water Quality Parameter Measurement

The WQP such as pH, DO, BOD<sub>5</sub> and COD were measured using pre-calibrated pH, DO, BOD and COD meters (Lovibond, Germany). The TSS and TDS were estimated following the standard methods [12]. Briefly, well-mixed samples were filtered, evaporated to dryness in a pre-weighed china dish and dried to constant mass at 180°C. The increase in dish weight represents the TDS which was measured using Eq. 5. Where A = mass of dried residue + dish (mg) and B = mass of dish (mg). For the measurement of TSS, a well-mixed sample was filtered in filter paper of known mass. The filter paper was kept for 1 h at 105°C in an oven, cooled, weighed and TSS was measured using Eq. 5.

$$TDS \text{ or } TSS = \left[\frac{A-B}{V(mL)}\right] \times 1000$$
(Eq. 5)

#### Toxicity Evaluation

Before toxicity evaluation, hydrogen peroxide was removed from treated samples by adding small amounts of  $MnO_2$  (< 1 mg/mL solution) [19]. After a reaction time of 1 h, the solutions were filtered and subjected to cytotoxicity tests and microbial load determination.

The onion bulbs of equal size were purchased from local vegetable market, Faisalabad, Pakistan. The bases of the bulbs were gently scrapped and root primordia were exposed to the test solution. Before transformation of bulbs in tested solution, bulbs were germinated in tap water and finally, best five were transferred to wastewater along with negative and positive controls. The germinated bulbs were kept in solution for 72 h and the tested samples were replaced with a gap of 6 h. Ultra-pure water was used as a negative control and methyl methanesulfonate (MMS) (Sigma-Aldrich) as positive control. The roots were harvested after period of 72 h and transferred to aceto-alcohol (1:3) solution. The root tips were hydrolyzed in 1N HCl at 60°C until they become soft and number of roots and their lengths were counted, measured and averaged [20].

For heamolytic assay, Powell *et al.* [21] procedure was adopted with slight modification. The sheep blood cells were gently mixed, poured into a sterilized polystyrene (15 mL) screw-cap tube (15 mL) and centrifuged for 5 min at 4000 rpm. The supernatant was decanted and the viscous pellets were washed three times with chilled sterile isotonic

phosphate buffer saline (PBS) solution (NaCl, 8 g/L; KH<sub>2</sub>PO<sub>4</sub>, 0.2 g/L; Na<sub>2</sub>HPO<sub>4</sub>, 1.2 g/L; and KCl, 0.2 g/L). The pH was adjusted to 7.4 using 1M NaOH and 1% HCl solution. The washed cells were suspended in 20 mL chilled, sterile PBS and the cells counted on a haemacytometer. The blood cell suspension was maintained on wet ice and diluted with sterile PBS to  $7.068 \times 10^8$  cells m/L for each assay and sample (20 µL) was aseptically poured into 1.5 mL appendrof tubes. For each assay, 0.1% Triton X-100 and PBS were used as the positive and negative control, respectively. Diluted blood cells suspensions (180  $\mu$ L) were aseptically poured into tubes and gently mixed. Appendrof tubes were incubated for 35 min at 37°C with agitation (80 rev/min). They were immediately placed on ice for 5 min and finally centrifuged for 5 min at 1310 x g. The supernatant was collected, placed in a sterile appendrof tube and diluted with 900  $\mu$ L chilled and sterile PBS. All tubes were maintained on wet ice after dilution and the absorbance was measured at 576 nm (BioTek, Winooski, VT, USA). All the samples were run in triplicate and results averaged. The percentage lysis of RBC was calculated using Eq. 6.; where A and B are representing absorbance of sample and triton X-100, respectively.

$$RBC \ lysis \ (\%) = \left[\frac{A}{B}\right] x 100 \tag{Eq. 6}$$

Brine shrimp (Artemia salina L.) eggs were hatched in artificial seawater. After 48 h of incubation at  $26-30^{\circ}$ C under constant aeration, the larvae (nauplii) were attracted to one side of the vessel with a light source, collected and transferred to the tested solution. Wastewater samples were diluted two times with artificial seawater and total volume was finally adjusted to 5 mL and 100 mL of suspension of nauplii containing 20 larvae. Later they were incubated for 24 h. The tubes were then examined under a magnifying glass and the numbers of dead nauplii were counted. Cyclophosphamide (10 µg/mL) was used as a positive control in all experiments, whereas artificial seawater was used as negative control. The percentage lethality was determined by comparing the mean surviving larvae of the test and controls [20].

Statistical Analysis

The data was analyzed using "R" statistical software (version 10.2) for construction of the

regression coefficients of the polynomial regression models for the optimization of independent parameters as well as response surfaces and contour plots.

#### **Results and Discussion**

#### Soap and Detergent Mills Wastewater Collection

The WQP of soap and detergent wastewater, before treatment, are given in Table-2. The measured pH, DO, COD, TDS and BOD were beyond the permissible limit and found in following range; pH 11.0-11.6, DO 1.7-2.01 mg/L, COD 1350-1500 mg/L, TDS 1289-1370 mg/L, TSS 700-790 mg/L and 550-700 BOD mg/L. After preliminary characterization, the wastewater samples were subjected to UV and gamma radiation treatment and various independent variables were optimized for maximum degradation. Finally, the effect of AOPs on cytotoxicity reduction was investigated where maximum degradation was observed using heamolytic, brine shrimp and allium сера cytotoxicity assays.

#### UV treatment procedure

The soap and detergent wastewater was treated by UV radiation and different independent variable (H<sub>2</sub>O<sub>2</sub> concentration, UV exposure time, pH, shaking and TiO<sub>2</sub> concentration) were optimized for maximum degradation of the pollutants. The UV treatment was carried out in the presence of 1.5-5.5% H<sub>2</sub>O<sub>2</sub> UV exposure time 40-120 min, (55 watt) at 6-12 pH and shaking speed 50-150 rpm in the presence of 3-7 g/L TiO<sub>2</sub>. Maximum degradation (95%) of pollutant in soap and detergent wastewater was achieved at different treatment levels. RSM analysis revealed that UV exposure time 100 min, TiO<sub>2</sub> concentration (5.93 g/L) at 4.39% v/v of H<sub>2</sub>O<sub>2</sub>, pH (6.50) and shaking speed (110 rpm) were the most effective treatment levels for maximum degradation of pollutant present in soap and detergent wastewater (Figs. 1 and 2). The polynomial relation between  $TiO_2$  concentration and wastewater is shown in Eq. 7, which showed that the TiO<sub>2</sub> concentration affected the degradation significantly (P < 0.005), where X is TiO<sub>2</sub> concentration and  $\epsilon$  is standard error.

$$D(\%) = 54.47(\pm 4.13) + 13.73(\pm 0.5)X_2 + \epsilon$$
 (Eq. 7)

	Table-2: Water c	juality para	meter of soap a	and detergent	industry before treatment.	
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S.No.	pН	DO	COD	TDS	TSS	BOD
	_	mg/L	mg/L	mg/L	mg/L	mg/L
1* (n=3)	11.6±0.63	1.8±0.6	1500±71.5	1165±58.25	700±82.7	600±35
2 (n=3)	11.5±0.53	1.7±0.15	1700±76.5	1370±68.50	750±62.62	700±40
3 (n=3)	11.0±0.58	2.1±0.7	1350±64.25	1289±64.45	790±37.00	550±41

Industry 1, 2 and 3 waste water was collected from Faisalabad, Sargodha and Gujranwala, respectively, COD-chemical oxygen demand, BOD-biological oxygen demand, TDS-total dissolved solids and TSS-total suspended solids



Fig. 1: Response surface and contour plots showing percentage degradation of soap and detergent wastewater subjected to UV treatment.



Fig. 2: Response surfaces and contour plots showing percentage degradation of soap and detergent wastewater treated by UV radiation. (A) Effect of pH, (B) Effect of shaking speed, (C) Effect of UV exposure time and (D) Effect of TiO<sub>2</sub>.



Fig. 3: Response surfaces and contour plots showing the WQP of soap and detergent wastewater treated by UV radiation; (A) COD, (B) BOD, (C) pH and (D) TDS



Fig. 4: Response surfaces and contour plots showing the TSS (A) and DO (B) of soap and detergent wastewater treated by UV radiation.

The WOP such as BOD, COD, DO, pH, TSS and TDS of soap and detergent wastewater were determined in order to evaluate the efficiency of treatment. The samples were treated at optimized conditions such as UV radiation for 100 min using  $TiO_2$  (3-7 g/L) and  $H_2O_2$  (4.39%) at shaking speed of 110 rpm, analyzed for WQPs and the results thus obtained are shown in figures 3 and 4. The reduction in COD and BOD were observed up to 48% and 52% for 3 g/L TiO<sub>2</sub>. By increasing the TiO<sub>2</sub> concentration to 5 g/L, the reduction in COD and BOD were increased up to 61% and 68%, respectively and no considerable reduction in COD and BOD was observed by further increasing the concentration of TiO<sub>2</sub> to 7 g/L. Through RSM analysis, it was observed that maximum reductions in COD and BOD took place for TiO<sub>2</sub> concentration of 3.66 g/L. The pH of the solution reduced significantly after UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> treatment, which is considered as good outcome of the treatment. Before treatment the pH of soap and detergent wastewater was in the alkaline range (11.0-11.6) and after treatment, it reduced (5.4-6.3). This indicates that degradation of pollutants has taken place since low molecular acidic species have been produced as a result of degradation associated with lowering in pH of the treated solution. Similarly, the TDS and TSS values were also reduced significantly and up to 45% and 46% reduction was observed for 3 g/L TiO2. However, TDS and TSS reduction was enhanced to 64% and 77% for 5 g/L TiO<sub>2</sub>. The radiation treatment in the presence of H<sub>2</sub>O<sub>2</sub> and TiO<sub>2</sub> increased the DO values to 66%.

## Gamma Radiation Treatment

The soap and detergent wastewater was treated by gamma radiation for the absorbed doses of 5, 10 and 15 kGy in presence of 1.5-5.5% H<sub>2</sub>O<sub>2</sub>. It has been observed that the degradation of contaminants present in wastewater is directly related to the water pollution load, which was found to be much higher than permissible limits. As a result of gamma radiation treatment in the presence of 1.5% H<sub>2</sub>O<sub>2</sub>, degradation of 41%, 57% and 69% was observed for the absorbed doses of 5 kG, 10 kGy and 15 kGy respectively. By increasing the  $H_2O_2$ percentage to 3.5%, the degradation increased up to 65%, 93% and 96% for the absorbed doses of 5 kGy, 10 kGy and 15 kGy, respectively. In the presence of 5.5% H<sub>2</sub>O<sub>2</sub>, the degradation did not enhance further. This is an indication of the fact that H<sub>2</sub>O<sub>2</sub> beyond optimized concentration was not found very effective for the degradation of contaminants (Fig. 5). Through RSM analysis, it was observed that 12.69 kGv absorbed dose and 4.65% H<sub>2</sub>O<sub>2</sub> were found better for maximum degradation of contaminants. The polynomial equation (Eq. 8,  $X_1 = H_2O_2$  concentration,  $X_2$  = gamma radiation absorbed dose and  $\epsilon$  = standard error) showed that gamma radiation absorbed dose and H<sub>2</sub>O<sub>2</sub> concentration both affected the degradation significantly (P < 0.005), however, the second degree term was insignificant in case of H<sub>2</sub>O<sub>2</sub> concentration. As a result of gamma radiation/H<sub>2</sub>O<sub>2</sub> treatment, the WQP improved significantly  $(P \le 0.005)$ . The reduction in COD is considered an important parameter to analyze the degree of pollution in terms of organic contents. The reductions in COD were 26%, 36% and 43% in samples exposed to the absorbed doses of 5 kGy, 10 kGy and 15 kGy in the presence of 1.5% H<sub>2</sub>O<sub>2</sub>, whereas it was 46%, 70% and 71% for 3.5%  $\rm H_2O_2$  concentration and by increasing the H<sub>2</sub>O<sub>2</sub> to 5.5% level, the reduction in COD value was observed to be insignificant (Fig. 6). It has been reported that COD reduction is directly related with degradation because when the complex organic molecules break down into small molecules upon treatment, the COD may reduce sharply [12]. Similar to the COD reduction, other WQPs such as BOD, TDS and TSS were improved and pH of the treated sample reduced (Figs. 6 and 7). The percentage reduction in BOD of soap and detergent wastewater sample subjected to the absorbed doses of 5, 10 and 15 kGy in the presence of 1.5% H<sub>2</sub>O<sub>2</sub> were 31%, 39% and 49% and by increasing the H<sub>2</sub>O<sub>2</sub> concentration to 3.5%, the BOD value decreased up to 52%, 72% and 75% for the absorbed doses of 5, 10 and 15 kGy. The reduction in TDS, TSS and increase in DO of treated sample was also found maximum for 3.5% H<sub>2</sub>O<sub>2</sub> concentration. The decrease in TDS was recorded to be 21%, 28% and 34%, while reductions in TSS were 26%, 37% and 44% for the absorbed doses of 5 kGy, 10 kGy and 15 kGy in the presence of 3.5% H<sub>2</sub>O<sub>2</sub> concentration, respectively. Under same conditions, the increase in DO was recorded to be 26%, 37% and 44% for 1.5% H<sub>2</sub>O<sub>2</sub> and 53%, 60% and 66% for 3.5% H<sub>2</sub>O<sub>2</sub> concentration (Fig. 7B). After treatment, the pH of treated samples reduced which is also a good indication of degradation of pollutants because the organic compounds are converted into low molecular weight organic acids and ultimately, the pH of treated sample decreased. Before treatment, the pH was highly basic and after treatment it was decreased and recorded to be in the range of 5.9-7.9.

 $\begin{array}{l} D \quad (\%){=} \quad -43.18(\pm 12.97 \ + \ 6.90(\pm 2.25)X_1 \ + \\ 41.35(\pm 5.14)X_2 \ - \ 0.60(\pm 0.21)X_1X_2 \ - \ 3.56(\pm 0.65){X_2}^2 \\ + \ \varepsilon \qquad (Eq. \ 8) \end{array}$ 

## Cytotoxicity reduction

For the evaluation of AOP effect on cytotoxicity reduction, cytotoxicity of raw and treated soap and detergent wastewater samples were compared using range of bioassays. The soap and detergent wastewater samples were highly cytotoxic in nature. The total bacterial and coliform populations were detected > 1 x  $10^6$  and >1 x  $10^5$ CFU (Table-3). However, after gamma radiation treatment, the microbial load was not detected and cvtotoxicity was also reduced significantly (P < 0.005). The cytotoxicity of wastewater samples subjected to the absorbed dose of 15 kGy in the presence of 3.5% H<sub>2</sub>O<sub>2</sub> was evaluated and results are shown in Table-2. The allium cepa tests showed a significant reduction in cytotoxicity and before treatment the number of root and root lengths were recorded in the range of 10-11 and 3.2-4.1 cm, respectively. After treatment, number of roots and root lengths increased up to 28.57-37.50% and 31.66-47.14%, respectively. The red blood cell lysis and shrimp Napoli death were recorded as 66% and 59% before gamma radiation treatment. However, after treatment, the lysis of red blood cell and shrimp death rates reduced considerably. The reduction in red blood cell lysis and shrimp Napoli death were recorded up to 84% and 90%, respectively. Similar to gamma radiation/H<sub>2</sub>O<sub>2</sub>, the UV/H<sub>2</sub>O<sub>2</sub>/TiO<sub>2</sub> treatment also reduced the cytotoxicity of soap and detergent wastewater (data not shown), however, the effect on toxicity reduction of radiation/H<sub>2</sub>O<sub>2</sub> was slightly higher as compared to UV/H<sub>2</sub>O<sub>2</sub>/TiO<sub>2</sub> treatment. It was revealed that both AOP reduced the cytotoxicity efficiently and bioassays are very sensitive to detect the cytotoxicity level before and after AOPs application.

Table-3: Cytotoxicity of soap and detergent wastewater before and after gamma radiation/H<sub>2</sub>O<sub>2</sub> treatment.

	Microbial test		Allium cepa		Heamolytic	Shrimp test
Before treatment	TBC	T coliform	RC	RL	Cell death	death
	CFU	CFU		cm	%	%
1* (n=3)	>1 x 10 <sup>6</sup>	1 x 10 <sup>5</sup>	$10 \pm 0.20$	3.2±0.04	69±1.1	61±0.60
2 (n=3)	>1 x 10 <sup>6</sup>	1 x 10 <sup>5</sup>	11±0.19	3.7±0.03	68±1.2	60±0.52
3 (n=3)	>1 x 10 <sup>6</sup>	1 x 10 <sup>5</sup>	$10 \pm 0.22$	4.1±0.03	67±0.9	59±0.61
PC			15±0.26	7.0±0.11	$100 \pm 0.0$	$100 \pm 0.0$
NC			$11 \pm 0.20$	3.0±0.02	0	0
After treatment						
1* (n=3)	ND	ND	16±0.78	5.9±0.30	11±0.54	8±0.33
2 (n=3)	ND	ND	17.0±89	7±0.37	12±0.65	7±0.21
3 (n=3)	ND	ND	14±0.65	6±0.22	11±0.50	6±0.16
Reduction (%)						
1* (n=3)	100	100	37.50	45.76	84.05	86.88
2 (n=3)	100	100	35.29	47.14	82.35	88.33
3 (n=3)	100	100	28.57	31.66	83.58	89.83

\*Industry 1, 2 and 3 wastewater was collected from Faisalabad, Sargodha and Gujranwala, respectively,

PC-positive control, NC-negative control, TBC-total bacterial count, T-total coliform, ACT-Allium cepa test, RC-root count, RL-root length, NR-not recorded, ND-not detected, n-sample seeded in triplicate

For heamolytic test, PC and NC were TritonX-100 (0.1%) and phosphate buffer saline, respectively

For ACT, PC and NC were distilled water and methyl methanesulfonate (MMS) (10 mg/L), respectively

For shrimp test, PC and NC were cyclophosphamide (10 µg/mL) and sea water, respectively



Fig. 5: Response surface and contour diagram showing percentage degradation of soap and detergent wastewater treated by gamma radiation for the absorbed doses of 5, 10 and 15 kGy in the presence of  $H_2O_2$  (1.5-5.5%).



Fig. 6: Response surfaces and contour plots showing (A) COD, (B) BOD, (C) pH and (D) TDS of soap and detergent wastewater treated by gamma radiation for the absorbed doses of 5, 10 and 15 kGy in the presence of H<sub>2</sub>O<sub>2</sub>(1.5-5.5%).



Fig. 7: Response surfaces and contour plots showing (A) TSS and (B) DO of soap and detergent wastewater treated by gamma radiation for the absorbed doses of 5, 10 and 15 kGy in the presence of H<sub>2</sub>O<sub>2</sub>(1.5-5.5%).

## Conclusions

The TiO<sub>2</sub>/UV/H<sub>2</sub>O<sub>2</sub> and gamma radiation/H<sub>2</sub>O<sub>2</sub> (AOPs) were found able to degrade the cytotoxic agent present in soap and detergent wastewater. The bioassay, Allium cepa, heamolytic and brine shrimp assays were found very sensitive biological tools to evaluate the AOP cytotoxicity reduction efficiency. For UV exposure time 100 min, TiO<sub>2</sub> concentration (5.93 g/L),  $H_2O_2$  (4.39% v/v) pH (6.50) and shaking speed (110 rpm) > 94%degradation was achieved and > 95% degradation was achieved for 12.69 kGy absorbed dose in the presence of 4.65%  $H_2O_2$ . Under these conditions > 75% and 70% reductions of BOD and COD were achieved. Both AOPs showed promising efficiencies for the degradation and cytotoxicity reduction of cytotoxic agent in soap and detergent wastewater. In future research, there is need to evaluate toxicity at all treated levels and the identification of radiolytic end product by using advanced spectroscopic techniques. So far, the AOP under investigation could possibly be used for the treatment of industrial wastewater to avoid environmental pollution [22-45], which is a serious environmental issue.

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