Transformation Products of Carbamazepine (CBZ) After Ozonation and their Toxicity Evaluation Using *Pseudomonas* sp. Strain KSH-1 in Aqueous Matrices

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Abstract Carbamazepine (CBZ) is an anti-epileptic and anti-convulsant drug widely used for the treatment of epilepsy and other bipolar disorders. Ozone as an advanced oxidation process has been widely used for the degradation of CBZ resulting in the formation of transformation products (ozonides). The present research aims to isolate and identify potential microorganism, capable of degradation of CBZ and its transformation products. The cell viability and cytotoxicity of pure CBZ and their ozone transformation products were evaluated using the cells of *Pseudomonas* sp. strain KSH-1 through cell viability assay tests. The cells metabolic activity was assessed at varying CBZ concentrations (~ 10–25 ppm, pure CBZ) and cumulatively for ozone transformation products. For pure CBZ, % cell viability decreases as CBZ concentration increases, while, in case of post-ozonated CBZ transformation products, the viability decreases initially and then increases upon exposure of ozone with a maximum cell viability of $97 \pm 2.8\%$ evaluated for 2 h post-ozonated samples.

Keywords Carbamazepine (CBZ) · Ozonation · Viability assay · MTT · 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

Introduction

In recent years, a lot of focus has been captured by active pharmaceutical ingredients (APIs) and pharmaceutical and personal care products (PPCPs) due to their worldwide occurrence, recalcitrant nature and ecological toxicity [1, 2]. Carbamazepine (CBZ) (Supplementary Fig. S1) is the most frequently used APIs for the treatment of epilepsy and other bipolar disorders [3]. CBZ, being recalcitrant, are highly resistant to conventional water and wastewater treatment processes and reported as an anthropogenic marker to indicate the pollution of the aquatic ecosystems [4, 5]. Due to its persistent nature and longer half-life periods in environment, CBZ finds its presence in several water bodies in both developing and developed countries [6]. This has led the society to take preventive measures for the degradation of this compound at its point source only. Furthermore, CBZ is difficult to biodegrade due to its structural complexity. However, few researchers, reported the biodegradation of CBZ using *Pseudomonas* sp. strain CBZ-4 isolated from the activated sludge at very low temperatures [7].

In past few decades, a lot of research has been focused on the production of value added materials and energy production in order to meet the global energy crisis in coming generations. In this context, wastewaters have also been utilized as an alternate source for production of energy [8]. Advanced oxidation processes (AOPs) have been extensively used as potential treatment processes for removal of pharmaceuticals from real and synthetic wastewater matrices [9, 10]. Ozone has been incorporated as potential AOP for the removal of pharmaceuticals as it provides a cleaner degradation compared to other conventional treatment processes [11, 12]. However, the partial breakdown of CBZ has been reported through direct/

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indirect ozone attack leading to the formation of transformation products (TP's), which sometimes exhibit higher toxicity than the original compound [13, 14]. Hence, it is necessary to evaluate the toxicity of the TP's formed after the treatment and to compare its toxicity with the parent compound, before their release in aquatic water bodies [15].

Therefore, to evaluate the toxicity, potential micro-organism capable of degradation of CBZ and its TP's has been isolated from the activated sludge samples. In this aspect, the aim of the work was divided into (a) enrichment and acclimatization of mixed microbial culture for screening of potential microorganisms capable of degradation of CBZ in a suspended growth reactor (SGR) [16]; (b) isolation and identification of potential microbial strain capable of CBZ degradation [7] and (c) evaluation of cytotoxicity of pure CBZ and cumulative effect of TP's on identified bacterial strain using cell viability tests (MTT assay). The cells metabolic activity were assessed by the conversion of MTT (yellow) into reduced formazon (purple) crystals by a NAD(H) dependent mitochondrial reductase enzyme.

To the best of our knowledge, the cytotoxicity evaluation of CBZ and their ozone TP's on *Pseudomonas* sp. has not been reported till date. Therefore, in the present study, ozone has been incorporated as a potential AOP for the treatment of CBZ followed by cell viability tests (MTT assay) on *Pseudomonas* sp. strain KSH-1 isolated from mixed microbial culture obtained from common effluent treatment plant (CETP) activated sludge. The % cell viability was evaluated both for pure CBZ (at varying dosage) and for ozone treated samples (increasing exposure time). Further, CBZ degradation pathway upon ozonation has been postulated and major oxidation/transformation products of CBZ has been identified by LC–ESI–MS and LC–MS/MS analysis. The results pertaining to these has been presented and discussed in this paper.

Materials and Methods

Chemicals, Reagents and Nutrient Media

CBZ (99% pure) and dimethyl sulfoxide (DMSO) were procured from Sigma-Aldrich (USA). Methanol (HPLC), isopropanol (99% v/v), formic acid, were of analytical grade purchased from Fisher Scientific (India). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was procured from Invitrogen, Thermo Fisher Scientific (India). All the other chemicals viz. K_2HPO_4 , KH_2PO_4 , $(NH_4)_2SO_4$, $MgSO_4 \cdot 7H_2O$, NaCl, NaOH, Na_2HPO_4 , CH_3COONa , NH_4Cl , $CaCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot H_2O$, $CuCl_2 \cdot 2H_2O$, $CoCl_2 \cdot 6H_2O$, etc.

were of reagent grade. Nutrient agar and nutrient broth were purchased from Himedia (India).

CBZ Synthetic Wastewater (SWW)

CBZ stock solution of 100 mg/L was prepared by dissolving pure CBZ in Milli Q water. A working solution of known concentrations (10, 15, 20 and 25 mg/L) were prepared for cytotoxicity studies using CBZ stock solution. Ozonation experiments were conducted using synthetic water having strength ca. 25 mg/L of CBZ.

AOP Reactor System for Ozone Experiments

AOP reactor system comprised of ozone generating system and borosilicate glass reactor vessel for homogeneous mixing of ozone. Ozone was produced through corona discharge method (air-cooled) using an ozone generator (Model INDOZ-20), Ozone Research & Applications India Pvt. Ltd. (ORAIP), Nagpur. Ozone dosage was determined by measuring the gas flow rate in inlet and outlet of the reactor using a BMT ozone analyser (Germany make). Unreacted ozone was continuously monitored in the outlet to ascertain that the reaction follows first order rate of reaction. All the ozonation experiments were conducted at room temperature by directly sparging ozone in CBZ synthetic wastewater (semi-batch mode) and with continuous stirring for homogeneous and efficient distribution of ozone [11]. The schematics of AOP reactor system have been represented in Fig. 1.

Enrichment Media and Culture of Microorganisms

The activated sludge comprising of mixed microbial culture was collected from the aeration tank unit of an operational pharmaceutical industry located in southern region of India. The mixed microflora was enriched in a suspended growth reactor (SGR) (2 L flask), by using artificial wastewater prepared as per the protocol mentioned by Vasiliadou et al. [16] with small modifications. The inoculum size of sludge liquor was 10% of the total volume of SGR. All the macro nutrients were added in same ratio, however, in case of trace elements, H_3BO_3 , $Na_2MoO_4 \cdot 2H_2O$ and KI were not added to SGR due to precipitation of media and side reaction. Further, in place of adding yeast extract, CH_3COONa was added to the media throughout the startup period in order to allow bacteria to adapt to the changing nutritional requirements. 50% of the SGR volume was replaced by fresh artificial wastewater containing CH_3COONa (1.8 g/L, 527 mgC/L) as only C-source.

The cell biomass growth was measured at every 4 h interval using spectrophotometric (OD_{600}) method. However, the dry cell mass was measured after each operating

Fig. 1 Schematic diagram showing experimental set-up for ozonation experiments (1) Oxygen concentrator, (2) ozone generating system (ORA IPL), (3) ozone analyzer BMT, Germany, (4) ozone destructor, (5) Magnetic stirrer unit, (6) Control panel system for the reactor, (7) Data acquisition system, (8) Cooling Water Inlet and outlet, (9) pH electrode, (10) Sampling port, (11) Magnetic stirring bar, (12) Sparger, (13) Head plate, (14) Ambient

cycle (24 h) by oven dried method. The cells after each cycle were harvested by centrifugation, washed with equal volume of distilled water (50 mL) to remove media interference, filtered and finally oven dried (105 °C, 8 h) and weighed. Significant biomass growth (ca. 355 mg/L) was observed after the startup period (4 days) and the inoculum (10% of the total volume) was transferred to a new SGR containing freshly prepared artificial wastewater with CBZ (5 mg/L) as sole C-source. The use of CBZ as sole C-source was necessary to acclimatize the culture and to develop microbial community capable of producing enzymes responsible for the biodegradation of such recalcitrant compounds. In order to acclimatize the culture, draw fill experiments were performed and after each operating cycle (24 ± 2 h), 1.4 L (70% volume) of the liquor was replaced with freshly prepared synthetic wastewater containing CBZ (ca. 5 mg/L) as sole C-source. The biomass concentration was found to increase slowly during first few cycles (days) and reached to about 500 mg/L (after 15 operating cycles) during the acclimatization period, which is higher than the biomass concentration during the startup period. The contents inside the SGR were stirred (300 rpm) and aerated continuously during the start-up and acclimatization period, in order to provide oxygenated environment for the growth of aerobic microorganisms.

Biodegradation of CBZ in SGR

After the acclimatization period, CBZ was decreased from 5 to 0.5 mg/L (~ 90% degradation, within 7 days), in

same SGR used for acclimatization of CBZ degrading microorganisms. CBZ biodegradation was further confirmed by performing additional experiments using fresh SGR but using inoculum of acclimatized microorganisms and ~ 80–90% CBZ biodegradation has been observed compared to initial CBZ concentrations.

Isolation and Identification of Potential Bacterial Cultures

The dominant bacterial cultures present in the SGR have been isolated using serial dilution technique on specific media plates. The specific media used for the growth of potential colonies comprised of minimal media (1×) (Composition: NH_4Cl = 0.23 g/L, KH_2PO_4 = 4.5 g/L, Na_2HPO_4 = 4.5 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ = 0.2 g/L, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ = 0.02 g/L) with CBZ (5 mg/L) as sole C-source and 2% Agar was added for solidification. The media was autoclaved for 15 min at 121 °C and have been used for preparation of specific media plates.

Specific media plates containing dominant colonies have been selected and streaked on fresh specific media plates by streak plate technique (four ways streaking) to obtain pure cultures. The pure cultures were characterized using morphological and biochemical tests. In parallel, the flask growth experiments of morphologically different colonies were performed and OD_{600} was measured. Most dominant and efficient CBZ-degrading bacterial strain (KSH-1) has been selected for DNA isolation and identification. The overnight grown pure bacterial culture was used for DNA isolation. The biomass has been centrifuged and lysed

using lysis buffer solution (400 μ L Tris–EDTA, 50 μ L of 10% sodium dodecyl sulfate (SDS), 50 μ L of (20 mg/mL) proteinase-K) and kept for incubation at 37 °C for 2 h. After the incubation period, an equal volume of PCI mix (phenol: chloroform: isoamyl alcohol, 25:24:1) have been added and properly mixed. The lysate were centrifuged and the DNA was precipitated using 90% ethanol. Precipitated DNA was dissolved in Tris–EDTA (TE) buffer. The purity and quantification of isolated DNA were determined by NanoDrop (ND-8000), Isogen Life Sciences.

Polymerase Chain Reaction (PCR) and 16S rRNA Gene Sequencing

Genomic DNA was isolated from the culture and 16 s rRNA gene was amplified using 27F (5'-AGAGTTT-GATCMTGGCTCAG-3') and U1492R (5'-TACGGY-TACCTTGTTACGACTT-3') universal primers. The PCR mix/100 μ L volume contained Taq buffer, Taq polymerase, deoxyribose nucleoside triphosphates (dNTPs), 0.2 μ M of each primer, and extracted DNA (50–100 ng). Amplification was carried using thermal cycler (GeneAmp 2700, Applied Biosystems, USA) with the PCR program: initial denaturation (95 °C, 10 min); 40 cycles of annealing temperature (94 °C, 1 min, 52 °C, 1 min, 72 °C, 1 min); and final extension (72 °C, 20 min). Resulting PCR product of about 1500 bp have been visualized by agarose gel electrophoresis (Supplementary Fig. S2) and purified by gel extraction using gel elution kit (Sigma Life Sciences). Further, purified PCR products were sequenced using Sangers Dideoxy DNA sequencing method. The sequenced FASTA file has been submitted to NCBI and microorganisms were identified using nucleotide BLAST tool search against the standard NCBI database.

Analytical Methods

General Parameters

The pH of pre-and post-ozonated samples was measured immediately using pH meter (Cyberscan Eutech 510, US made). UV scanning has been performed using UV-1800 spectrophotometer (Shimadzu; spectral bandwidth 1 nm) while the Total Organic Carbon (TOC) content of the samples were measured using Shimadzu TOC-L instrument equipped with an ASI-V autosampler. CBZ concentration in pre-and post-ozonated samples were determined using Waters HPLC (USA) equipped with Waters 2998 Photodiode array detector. High strength silica (HSS) C-18 column, (4.6 mm $\times$ 250 mm, 5 μ m, Waters, USA) has been used for the separation of CBZ. The samples were run in isocratic mode with flow rate of 1.5 mL/min. The mobile phase was prepared by mixing Milli-Q deionized water and

methanol (v:v = 30:70) and 0.1% formic acid. The injection volume of each sample was 20 μ L and the column oven temperature was maintained at 25 °C. All the samples were filtered using 0.22 μ m syringe filters prior to analysis.

LC–MS/MS Analysis

The major transformation products of CBZ have been identified using UPLC–ESI–MS system (Waters, USA) equipped with Thermo accucore C-18 LC column (100 mm $\times$ 3.0 mm, 2.6 μ m) and Waters UPLC–TQD mass spectrometer (ESI–MS, APCI–MS, LC–MS/MS) with the mass range of 100–2000 Da. However, the quantification of transformation products/intermediates was not possible due to the unavailability of commercial standards of transformation products or intermediates.

The mobile phase used for the separation of TP's consisted of (A) Acetonitrile and (B) 5 mM NH_4Ac . The gradient mode of elution was applied as follows: 0–3 min, 20% A: 80% B; 3–6 min, 40% A: 60% B; 6–8 min, 70% A: 30% B; 8–10 min, 80% A: 20% B; 10–12 min, 20% A: 80% B. The flow rate was kept constant at 0.35 mL/min with a flow ramp rate of 0.45 min. The ESI was operated with following parameters: spray voltage 3.5 kV, source temperature 120 °C, desolvation temperature 350 °C, MS scan mode—SIR (selected Ion recording)/MRM (Multiple reaction monitoring). For mass spectra acquisition, the chromatograms were obtained in total ion current (TIC) mode with a scan range of m/z 50–350.

Bacterial Growth and Cell Viability Assay

For bacterial growth curve study, the bacterial culture was allowed to grow overnight in minimal media solution in a shaking incubator set at 37 °C, 120 rpm. Overnight grown bacterial cells (from exponential phase) have been used as inoculum for carrying out flask experiments. Two different sets of flask experiments were performed, one at varying CBZ concentrations added to the minimal media to achieve CBZ concentrations (10 ppm, 15 ppm, 20 ppm, 25 ppm), and other set containing minimal media but, supplemented with equal volumes of ozone treated samples (Initial and post-ozonated for 10 min, 30 min, 60 min and 120 min). All the flasks were autoclaved (121 °C, 15 min), inoculated (100 μ L, overnight grown) and incubated at 37 °C with continuous shaking (120 rpm). The growth (OD_{600}) of bacterial culture was measured at every 4 h intervals using UV–visible spectrophotometer.

Bacterial cell viability was determined using MTT assay (Supplementary Fig. S3) [17]. The bacterial cultures (950 μ L each) were collected in centrifuge tubes (capacity 1.5 mL) at intervals of every 4 h from each experimental flask. MTT (1.28 mg/mL, 30 μ L each) and glucose (0.5%,

20 µL each) has been added to the tubes and incubated at 37 °C, 1 h in dark conditions. Cells were then harvested, centrifuged (at 10,000 rpm, 5 min) and the supernatant (comprising of growth medium and MTT solution) was decanted. The pellet comprising of formazan crystals were finally suspended in dimethyl sulfoxide (DMSO) (250 µL) solution to dissolve the crystals and absorbance ($A_{550\text{ nm}}$) was measured corresponding to each well using Tecan infinite 200 PRO microtiter plate reader [18]. All the experimental assays have been carried out in triplicates ($n = 3$), and the results were presented in terms of % cell viability (average $\pm$ SD). The cell viability has been determined using following equation:

$$\text{Cell Viability (\%)} = \frac{\text{Test} - \text{PC}}{\text{VC} - \text{PC}} \times 100 \quad (1)$$

PC positive control (minimal media with C-source + 1% inoculum); VC vehicle control (minimal media + inoculum, C-source absent), Test samples (minimal media with varying CBZ as C-source + 1% inoculum each).

Results

Transformation products of CBZ after ozone treatment

Pre- and post-ozonized CBZ wastewater was subjected to UV scanning to gain preliminary insights into the degradation of CBZ along with the formation of ozone transformation products of CBZ (Supplementary Fig. S4). For ozone treated synthetic wastewater samples, a continuous decrease in absorbance at $\lambda_{285\text{ nm}}$ (corresponding to CBZ) has been observed which indicates the possible degradation of CBZ. However, the formation of transformation products was confirmed by using HPLC (Supplementary Fig. S5) and LC–MS/MS analysis (Supplementary Fig. S6 and S7). From Supplementary Fig. S5 it is evident that new peaks have been formed as the ozonation proceeds. These peaks were evolved at retention times of $R_t = 2.6\text{ min}$ (Supplementary Fig. S5 b, c) and $R_t = 2.2\text{ min}$ (Supplementary Fig. S5 d, e) compared to the initial ($R_t = 2.8\text{ min}$ for CBZ) (Supplementary Fig. S5 a) and corresponds to the possible transformation products. However, the possible TP's were confirmed and identified on the basis of m/z ratio by using LC–MS (Supplementary Fig. S6 a–d) and LC–MS/MS analysis (Supplementary Fig. S6 e–f, S7). Three major transformation products were namely 1-(2-benzaldehyde)-4-hydro-(1H,3H)-quinazoline-2-one (BQM) (Supplementary Fig. S6 g); 2-(2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)benzaldehyde (BQD) (Supplementary Fig. S6 f); and 2-(2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)benzoic acid (BaQD) respectively. Further, on the basis

of above identified TPs, mechanism of CBZ degradation pathway have been postulated and presented in Fig. 2, while pH, CBZ concentration and % TOC removal with time of ozone treatment has been presented in Fig. 3.

Proposed Degradation Pathway of CBZ

Ozone can react with CBZ (Fig. 2, st. I) via two distinctly different mechanisms i.e. direct attack (O_3) or indirect attack (formation of $^{\circ}\text{OH}$ radical) [14, 19]. However, when ozone is surplus (non-limiting) it acts by direct attack on $\text{C}^4 = \text{C}^5$ position of the CBZ ring (criegee mechanism), thereby forming intermediates called 'ozonides' (Fig. 2 st. II and III) which are highly unstable and rapidly rearranges and transforms into more stable products (Fig. 2 st. VI, VII and VIII) through subsequent unstable intermediates (Fig. 2 st. IV, V). The stable intermediates VI, VII and VIII (Fig. 2) were identified as BQM, BQD and BaQD respectively. Similar transformation products were also identified and reported by McDowell and co-workers using GC–MS, LC–ESI–MS/MS and ^{13}C -NMR techniques [13].

Bacterial Growth, Cell Viability and Cytotoxicity Assay

The cytotoxicity of pure CBZ and cumulative cytotoxicity of its major ozone transformation products have been evaluated using *Pseudomonas* sp. strain KSH-1 (GenBank accession no. KX554414) isolated from activated sludge samples. The bacterial growth (absorbance 600 nm) was monitored both for pure CBZ and ozone treated samples at every 4 h intervals (Supplementary Fig. S8 a, c). It was observed that at any given time interval, for pure CBZ, the bacterial growth ($\text{OD}_{600\text{ nm}}$) decreases as CBZ strength increases, with maximum growth observed in control samples containing sodium citrate as alternative C-source (Supplementary Fig. S8 a). This is mainly because of the structural complexity and recalcitrant nature of CBZ towards biological degradation. However, no significant change on growth has been observed for ozone treated samples (Supplementary Fig. S8 c).

Further, absorbance (550 nm) was also measured for both pre- and post-ozonated CBZ samples to detect the formation of purple coloured formazan crystals (MTT assay) Supplementary Fig. S8 b,d). Production of formazan complex is directly coupled with the cellular metabolic activity of the bacteria. Moreover, % cell viability of *Pseudomonas* sp. strain KSH-1 was evaluated after 24-h exposure to pure CBZ and ozone treated CBZ samples (Eq. 1). All the experimental analyses have been conducted in triplicates and the results were represented as average $\pm$ standard deviation (error bars). It has been observed that the % cell viability decreases with the increase in CBZ concentration with maximum viability (98.5%) detected for

Fig. 2 Proposed degradation pathway of CBZ after ozone treatment

10 ppm CBZ and minimum viability (57.2%) pertaining to 25 ppm CBZ (Fig. 4a). Further, the % cell viability of *Pseudomonas* sp. decreases with ozonation in the beginning (60.2–43.5% within 30 min) and then increases as ozonation progresses further (43.5–97%) (Fig. 4b) with maximum cell viability of $97 \pm 2.8\%$ observed in case of 2 h post ozonated samples.

Discussion

The formation of three major transformation products viz. BQM ($m/z = 251$), BQD ($m/z = 267$) and BaQD ($m/z = 283$) were the result of addition of subsequent oxygen atoms (by ozone) to the parent compound CBZ ($m/z = 236$). However, the gain in the molecular weights were

Fig. 3 pH, % TOC removal and CBZ concentration profile with time of ozonation (min)

Fig. 4 MTT assay showing % cell viability of **a** pure CBZ at varying concentrations and **b** ozone treated CBZ with increasing time of ozone exposure (Error bar represents $\pm$ standard deviations whereas, 'n' represents number of times experimental analysis conducted, and n = 3 viz. triplicate experimental analysis)

m/z = + 14, +30 and +46 respectively, which is found to be one or two units less than the exact molecular weight of oxygen atoms. This could be explained by the de-protonation effect during the double bond formation and may also be attributed to the internal cyclization/rearrangement of aromatic rings. The principal reason behind the initial decrease in cell viability (within first 30 min) has been

ascribed mainly due to the cumulative toxicity of above three TP's (BQM, BQD and BaQD).

Further, the fragmentation pattern after LC–MS/MS reveals the formation of daughter molecules of BQM, BQD and BaQD having low mass range. These lower mass fragments have m/z in the range 104, 129, 147, 196, 224 (Supplementary Fig. S6 e–f) due to the loss of molecular entities corresponding to loss of water, carbonyl or cyanide groups. These lower molecular weight (m/z) compounds have also been reported by Hubner et al. [14]. Moreover, these transformation products were simultaneously present in the aqueous matrices during the course of ozonation experiments and the overall toxicity has been attributed to the cumulative effects of all the fragmentation products dominantly present at a particular time period. However, as the ozonation proceeds, these TP's further decompose into simpler compounds, thereby increasing the cell viability of the bacteria.

The reduction in toxicity of 2 h post-ozonated samples towards *Pseudomonas* sp. strain KSH-1 could be attributed to the decrease in strength of TP's and/or dissociation of aromatic ring structures leading to the formation of linear chain compounds. However, the TP's could not be quantified due to non-availability of commercial standards for BQM, BQD and BaQD.

Conclusion

Ozone as an advanced oxidation process (AOP) has been effective in the degradation of CBZ from synthetic wastewater. BQM, BQD and BaQD were identified as major transformation products (TP's) of CBZ upon ozonation and pathway for mechanism of action of ozone on CBZ has been postulated. These transformation products were present together during the ozone treatment process and therefore the toxicity caused by these TP's on *Pseudomonas* sp. strain KSH-1 was due to the cumulative effect of all the three TP's. The cell viability tests (MTT assay) have been conducted for the toxicity evaluation of TP's and *Pseudomonas* sp. strain KSH-1 was used as model microorganism for the assay. It has been observed that the cell viability decreases in the beginning (43.5% viability with 30 min ozonated samples). However, the final degradation products of CBZ after 2 h ozonation do not exhibit cytotoxicity on *Pseudomonas* sp. strain KSH-1 and exhibit a cell viability of $97 \pm 2.8\%$ with the ozone treated samples.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

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