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DEVELOPMENT OF A HIGH-INTENSITY, PULSED-PLASMA, GAS-DISCHARGE TECHNOLOGY FOR DESTRUCTION OF HAZARDOUS AQUEOUS ENVIRONMENTAL MICROPOLLUTANTS

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Abstract: The aim of this study is to investigate the development and optimization of a high-intensity, pulsed plasma, gas-discharge (PPGD) system as a novel environmental decontamination approach for treating unwanted microbial and chemical micropollutants. This PPGD system produces multiple short-lived decontaminating properties in the treatment chamber, which includes ozone, acoustic shock waves, UV-light and pulsed electric fields. Findings demonstrated that PPGD effectively inactivated a broad range of microbial pathogens including antibiotic-resistant bacteria and also significantly reducing phenol in treated samples ($p< 0.05$). However, HPLC analysis revealed that application PPGD produced a range of break-down by-products in phenol-treated samples, which exhibited significant ecotoxicological effects as demonstrated by use of Microtox™ assay. Greater ecotoxicological were observed from samples post PPGD treatments compared to that of the untreated-Phenol control samples ($p<0.05$). Shorter-exposure periods to PPGD treatment produced sub-lethal conditions for survival of test microbial pathogens, which were underestimated compared to enumeration of similarly PPGD-treated samples by conventional agar plate counts ($p<0.05$). While PPGD was shown to be an effective electro-technology for reducing or removing environmental micropollutants in water, it must be combined with other decontamination approaches in order to mitigate against undesirable toxicological end-points produced during treatments.
Keywords: Pulsed Plasma Gas Discharge, Environment, Microbial Inactivation; MRSA, Ecotoxicology

I. INTRODUCTION

There is growing international concern about the release of unwanted pharmaceutically-active compounds (PhACs) into the aquatic environment as these are not effectively removed or eliminated at the wastewater treatment plant level [1, 2]. Certain pharmaceuticals and other chemicals, which can be harmful even in very low concentrations, can pass easily through sewage treatment plants. These chemicals can be detected in surface waters, groundwater and drinking water [2–4] by both conventional grab and the emerging passive sampling techniques, the latter been adopted where conventional measurement approaches are not

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sufficiently sensitive for detection [2]. Commensurately, there also concerns about the resilience of antibiotic bacteria and their antibiotic resistance genes to conventional wastewater treatment approaches [5]. Therefore, additional wastewater treatment measures, such as ozonation and other advanced oxidation processes (AOP), have been investigated for the elimination of priority and emerging contaminants of concern across EU countries as end-of-pipe solutions [2]. Until recently, environmental regulations worldwide had not required explicit testing for any PhACs in water bodies. However given the growing concern about contamination of the aquatic environment with these compounds, legislation has recently begun to acknowledge this potential problem. The Water Framework Directive (WFD, 2000/60/EC) is an overarching piece of European environmental legislation aimed at protecting and improving water quality throughout the EU. It is clear that the WFD, which is committed EU Member States to achieve good qualitative and quantitative status of all water bodies by 2015, has still some work to do.

PhACs in the aquatic environment primarily originate from use in human medicines, however certain classes are also heavily used in veterinary practices (e.g. anti-inflammatory drugs, antibiotics) [3, 4]. A large number of PhACs have been detected in WWTPs influents and effluents and surface, ground and drinking water worldwide in recent years [6-8]. In fact it is now established that throughout the developed world, PhACs are ubiquitous at µg to ng per litre levels in the aquatic environment [7], although the concentrations of specific compounds depend on usage patterns in different countries and can vary temporally [9]. The impacts of chronic exposure to trace concentrations of many PhACs on wildlife and human health may be severe (e.g Verlicchi et al [9]), thus it is critical to limit as much as possible the concentrations of this class of contaminants in our waterways. Certain PhACs can specifically impact the endocrine system of humans or wildlife; such chemicals are part of a larger classification of emerging pollutants known as endocrine disrupting chemicals (EDCs). Much of the growing interest in this field of research stems from fears that chronic exposure to EDCs (in bathing or drinking water, for example) may be linked to adverse human health conditions such as declining male fertility, birth defects, and breast and testicular cancer [7]. Furthermore negative impacts of EDCs exposure on wildlife may include severe consequences such as feminisation in fish 2007). Similar to PhACs as a whole, EDCs are mainly thought to be transported into the aquatic environment via incomplete removal at WWTPs [7].

An alternative or complementary advanced oxidation process (AOP) to the treatment of drinking and wastewater includes the application of high voltage pulses to gas-injected test liquids (or PPGD), which results in the formation of a plasma that causes free radicals such as dissolved ozone and hydrogen peroxide, free electrons, ultraviolet light (UV), acoustic shock waves and electric fields at levels between 10 and 50 kV to be generated in the test liquids [10-12]. Researchers have reported that pulsed electric discharge treatment can reduce dyes, phenol and aniline in aqueous solutions [13 – 16], however, there is a dearth of information regarding the ecotoxicology of PPGD-treatment of micropollutants along with possible survival of sub-lethally injured PPGD-treated microbial pathogens. This constitutes the first study to report on the ecotoxicity of PPGD-treated phenol combined with efficacy for destruction of antibiotic-resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA).

II. METHODOLOGY

Pulsed Plasma Gas-Discharge Treatments

The pulsed-plasma gas-discharge (PPGD) system (Samtech Ltd, Glasgow, UK) consisted of a prototype HV pulse generator and treatment chamber for low-temperature liquid decontamination which was described previously by Hayes et al. [12] with modifications. The main components were the high voltage power supply, a set of charging resistors, a trigger generator linked to a corona stabilised switch (CSS) and the pulse generator. This PPGD system produced multiple short-lived biocidal properties in the treatment chamber that includes ozone, acoustic shock waves, UV light and pulsed electric fields as described previously [10]. The PPGD test liquid was prepared by combining 95 ml of dH_2O combined with 5 ml of 0.1M Phosphate Buffered Saline (PBS). The provided high permittivity (ε=80.1) and low conductivity (1000 µS/cm) supported discharge stability and reduced osmotic stress towards test organisms. The volume of the treatment test liquid was set at 100 ml as this yielded at discharge gap of 5 mm between the electrodes and the surface of the sample that produced a strong and consistent HV discharge. Within the treatment chamber the PPGD was generated via a multiple needle electrode configuration that was found to produce a positive streamer type corona discharge. The electrodes were made of two materials: the upper HV electrode and needle arrangements comprised stainless steel BS316S11 (predominately iron (Fe) with 16-18.5% chromium (Cr), 11-14% nickel, 2-5% molybdenum and a mixture of 2% manganese (Mn), 1% silicon, 0.045% phosphorous, 0.03% sulphur, and 0.03% carbon) with the upper earth electrode comprised aluminium allow 6082 (predominantly aluminium (Al)

with 0.7-1.3% silicon, 0.4-1% Mn, 0.6-1.2% magnesium, a mixture of 0.5% Fe, 0.25% Cr, 0.2% zinc, 0.1% copper and 0.1% titanium).

The PFN charging voltage and pulse frequency were mainly kept at 20 kV and 10 pps respectively for ≤15 min during this study to avoid internal arcing on the CSS caused by molecular breakdown of sulphur hexafluoride (SF_6) insulation gas that occurred during increased charging voltage and pulse repetitions. All studies were performed using oxygen at a flow rate of 2L/min. The pulse waveform was monitored using a digital oscilloscope (Tektronic TDS 3022).The 100 ml test liquid awith and without *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Campylobacter jejuni, Bacillus subtilis* endospores at populations of $\leq 10^8$ CFU/ml was aseptically separately transferred into the PPGD treatment chamber as per method of Rowan et al. [10]. Temperature and pH were monitored by using an Eutech® CyberScan pH 510 pH/mV meter with automated temperature control adjustment (Thermo Fischer Scientific, UK). The dissolved ozone (O_3) concentration of gas-discharge treated water was analysed via the dipropyl-p-phenylenediamine (DPD) colorimetric method (Spectroquant® 1.00607, Merck Chemicals, VWR, Ireland), which detects dissolved O_3 within the concentration range 0.01-4.00 mg/l.

Phenol (Sigma Aldrich, Ireland) was PPGD-treated in 100ml dH_2O at 100ppm. High Performance Liquid Chromatography (HPLC) was used to analyze and quantify the concentration of Phenol in the samples before and after treatment. The chromatographic system was composed of a Shimadzu LC-6A HPLC pump, a Waters 486 UV/VIS tunable absorbance detector and a Shimadzu C-R6A chromatopac for system and data management. The mobile phase consisted of methanol in distilled water (ratio 60:40). The standards for the calibration curve were prepared from HPLC grade Phenol purchased from Sigma and diluted in mobile phase. The final standard concentrations were obtained by appropriate quantitative dilutions from the stock solution using the mobile phase as the diluent. A Phenomenex Sphere Clone (250 x 4.6mm, 5 micron) column was used with a flow rate of 1ml/min. The concentration of treated phenol was calculated using linear regression analysis of peak areas determined from the calibration curve (Figure 1).

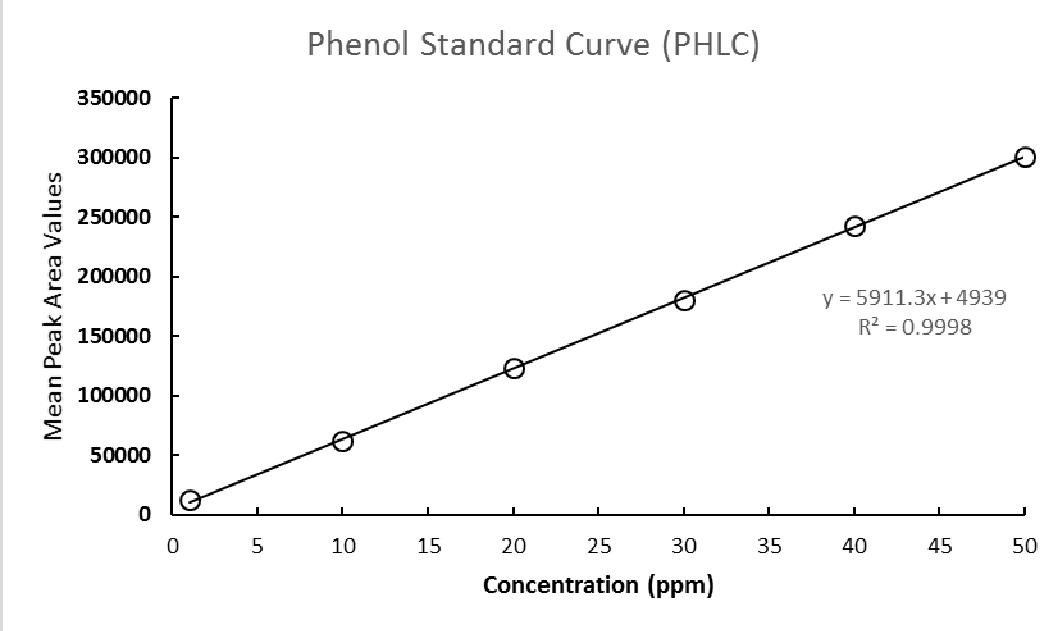

Figure 1 HPLC standard curve for phenol using mean concentration values.

Use of epifluorescence microscopy and fluorescence redox probes to enumerate respiring cell numbers after PPGD treatments

Epifluorescence microscopys and the fluorescent redox probes 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) and 4',6-diamidino-2-phenylindole (DAPI) were used to investigate respiratory activity in test strains according to previously described procedures, with modifications [17]. One-millilitre cell suspensions were harvested by centrifugation (4ºC for 10 min at 3,000 X *g*) and washed three times with PBS. Experimental and control preparations were resuspended in 300 µl of 5mM CTC (Polysciences, Inc, St. Louis, Mo.) and incubated in microaerophilic (for *Campylobacter* only) or aerobic environment for 1.5 h in the dark at 20ºC with agitation (200 rpm). After incubation, experimental and control preparations, and dilutions thereof, were counterstained for 8 min at 20ºC with 5 µg of DAPI (Sigma, St. Louis, Mo.) ml^{-1} and samples were transferred to a Petroff-Hausser counting chamber for enumeration. Counterstaining with the DNA-binding DAPI allowed concurrent determinations of total (i.e., viable plus nonviable) bacteria and viable (i.e., only cells exhibiting red CTC-formazan fluorescence) bacteria. Epifluorescence observations of CTC-treated preparations were viewed using a blue 420 to 480-nm excitation filter (combined with a 580-nm dichromic mirror and a 590-nm barrier filter) in a Nikon Optiphot microscope. CTC-and DAPI-stained bacteria in the same preparation were viewed simultaneously with a 365-nm-excitation filter, and emission filter and a 400-nm-cutoff filter. Stained cells were distinguished from non-specific reactions by overlaying the fluorescence and phase-contrast

images. Counts were determined from five randomly selected squares on the chamber etched-grid in triplicate experiments and results were expressed as the log number of corresponding bacteria per millilitre of sample. Analysis of variance, balanced model (Minitab software Release 14, Minitab Inc., State College, PA) was used to compare the effects of PPGD treatments on microbial inactivation. Experiments were replicated three times with duplicate treatments in each replication, and results are reported as means ± standard deviations. Significant differences were reported at the 95% ($P < 0.05$) and confidence interval.

Ecotoxicology

The Microtox™ bioassay is a standardised toxicity system using a luminescent bacterial species, *Vibrio fisheri*, as the toxicity indicator. Microtox™ determines the acute toxicity of aqueous samples by measuring the changes of light produced naturally in samples exposed to bioluminescent bacterial under standard conditions. This change in light output and the concentration of the toxicant produces a dose/response relationship which enables the calculation of an IC_{50} value after 5, 15 and 30 mins, the concentration which inhibits 50% of the bacteria. The Microtox™ test was carried out as per the suppliers' standard operating procedure (Azur Environmental, 1992). Each test was carried out in triplicate on a Microtox™ 500 analyser (Strategic Diagnostics Incorporation Europe). Positive control tests were performed using the reference toxicant potassium dichromate (K2Cr2O7) as recommended in the kit manuals. The acute toxicity data was obtained and analysed using the MicrotoxOmni software (SDI Europe, Hampshire, UK). Data for the ecotoxicological analysis PPGD treated test samples are presented as per Garvey et al. [18]. Differences between treatments and controls were tested by analysis of variance (ANOVA) and Dunnett's test. Statistical analysis for the Microtox™ test was performed using the Microtox ''Chronic Toxicity Testing DOS software'', in accordance with guidelines provided by the US Environmental Protection Agency (1994).

III. RESULTS

Findings showed that application of PPGD inactivated ca. 8 log orders of all test organisms at 15 min (20 kV/cm, 10 pps, O_2 flow rate of 2 L/min) (Figure 2). The order of increasing sensitivity to PPGD treatment was *Bacillus subtilis* endospores, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Escherichia coli* and *Camplylobacter jejuni* (Figure 2). The antibiotic resistant pathogen MRSA was particularly sensitive to PPGD treatments. Use of vital redox staining technique revealed that enumeration of PPGD-treated vegetative test bacteria by conventional agar -plate counts

consistenty underestimated survivors (Table 1) at shorter exposure durations (5 mins). *Bacillus subtilis* endospores were not affected by this process. The pH value of PPGD-treated samples increased slightly in an almost liner fashion with highest value of 8.12 observed after 15 mins. The concentration of ozone measured after 15 mins PPGD treatment was 1.1 mg/L. PPGD treatment reduced to phenol from 50 ppm to less than 1 ppm after 15 mins treatment at 20 kV, 10 pps using O_2 flow fate of 2 L/min (Figure 3) as measued by HPLC. The HPLC chromatogram shows the formation of numerous by-products (Figure 3b); however the phenol peak (at 5.7 minutes) was greatly reduced (Figure 3a). The mean peak area of 50 ppm phenol in untreated samples was 300,820 (±1098), while the mean peak area of the PPGD treated sample was 8,626 (±364) (Figure 3b). Using regression analysis the mean peak area of the treated sample correlates to less than 1ppm of phenol in the PPGD treated samples. Ecotoxicology findings demonstrated that PPGD-treated phenol samples exhibited greater toxicity to that of untreated phenol control as measured by Microtox™ assay (Figure 4). This toxicological effect was more pronounced after extended the Microtox™ assay from 5 mins (Figure 4) to 15 mins before determinations (Figure 5).

Table 1. Enumeration of test bacterial cell numbers by respiratory staining (RS) and colong forming units by direct plate count (PC) after 5 mins PPD treatment (20 kV, 10 pps, flow rate O_2 at 2 L/min)

Test bacteria	Untreated (PC)	Untreated (RS)	PPGD (PC)	PPGD (RS)
C. jejuni	7.8 (±0.2)^{A}	7.6 (±0.3)^{A}	4.1 (±0.5)^{D}	4.9 (±0.6)^{C}
P. aeruginosa	7.7 (±0.3)^{A}	7.1 (±0.2)^{A}	4.1 (±0.3)^{D}	5.7 (±0.4)^{C}
E. coli	7.6 (±0.2)^{A}	7.1 (±0.2)^{A}	4.0 (±0.4)^{D}	5.1 (±0.5)^{C}
MRSA	7.8 (±0.3)^{A}	7.5 (±0.3)^{A}	4.7 (±0.4)^{D}	5.2 (±0.5)^{D}
B. subtilis (spores)	7.3 (±0.1)^{B}	NA*	4.6 (±0.4)^{D}	NA

Values followed by the same upper case letter in separate columns do not significantly differ at the 95% confidence interval (P<0.05)

*Not applicable, as CTC-DAPI respiratory staining (RS) does not stain endospores.

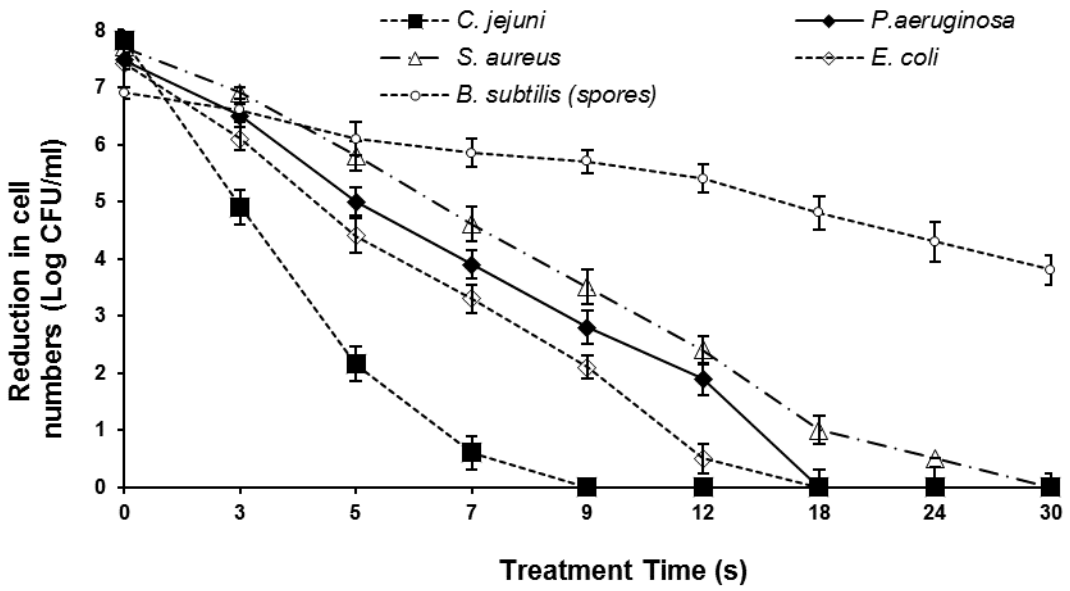

Figure 2. Influence of PPGD-treatment (15 mins, 20 kV, 10 pps, O_2 at 2L/min) on the inactivation of test bacteria suspended in dH_2O at 4°C

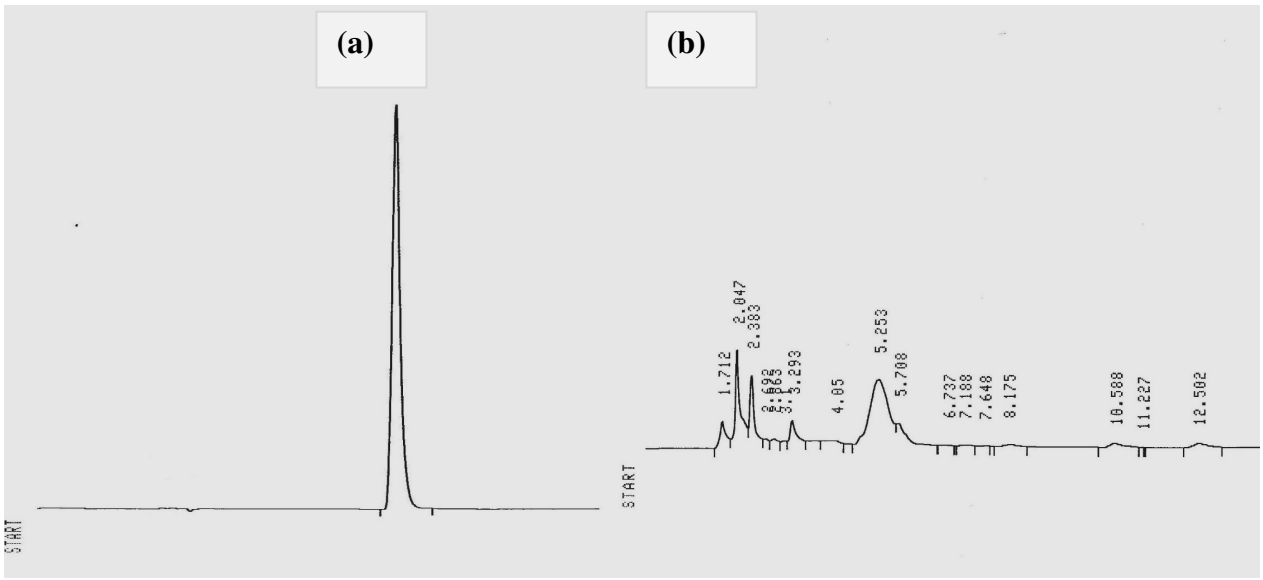

Figure 3. HPLC chromatograms of untreated phenol (a) and PPGD-treated (b) phenol samples after 15 mins exposure at 20 kV, 10 pps, flow rate of O_2 at 2L/min

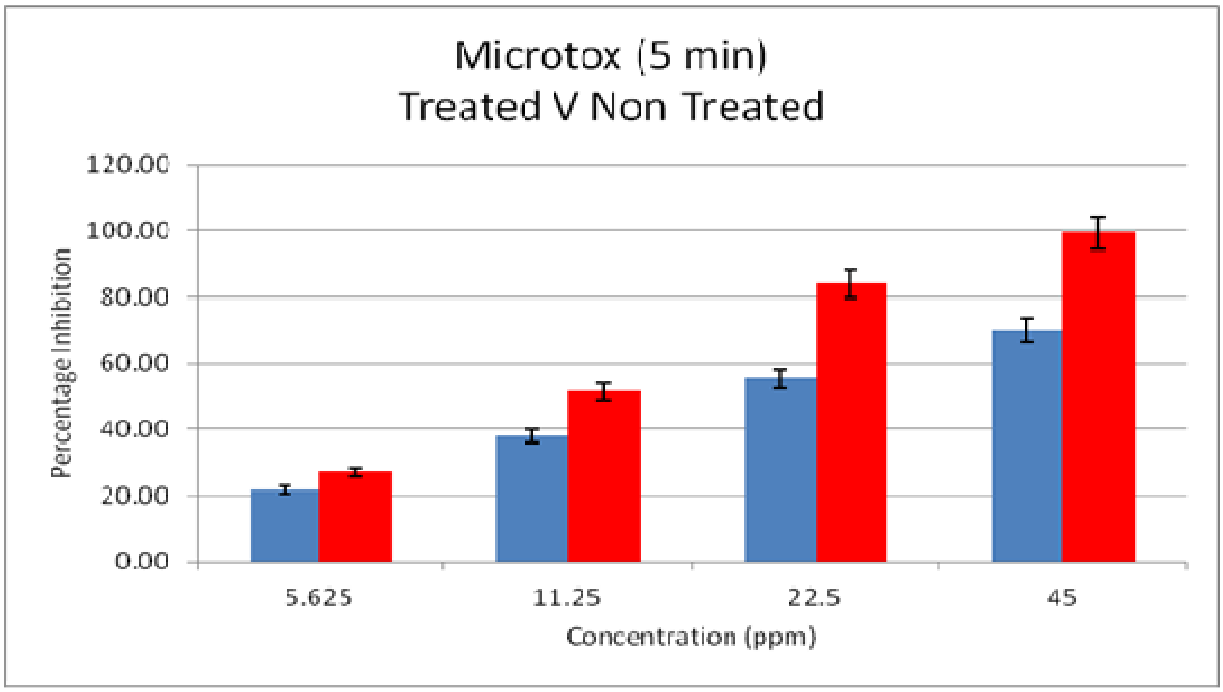

Figure 4: Comparison of the toxicity of PPGD-treated phenol (red) versus non treated phenol (blue) by Microtox® at 5 mins

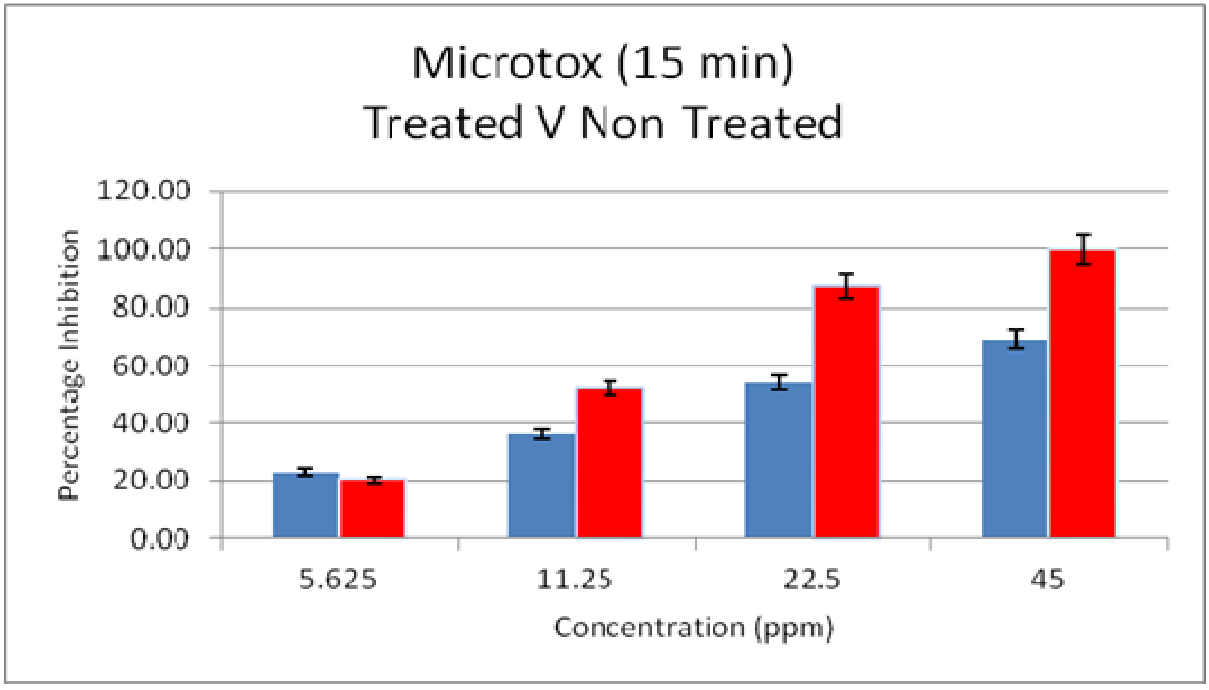

Figure 5: Comparison of the toxicity of PPGD-treated phenol (red) versus non treated phenol (blue) by Microtox® at 15 mins.

IV. DISCUSSION

This study has demonstrated that PPGD treatment effectively reduces a broad range of problematic bacteria including the recalictrant endospores that are produced by *Bacillus* species under aerobic conditions. However, use of shorter durations of PPGD is less biocidal and creates conditions that promotes the generation of sub-populations of treated cells that may be still viable but not culturable as defined by growth on on conventional agar plates. The underestimation of microbial suvivors to non-thermal processes, such as high intensity pulsed light [19, 20] and plasma discharge processes [10] has been prevously reported. The viable but non-culturable state in environmentally stressed microbial speceis has been reviewed by many resarchers [17, 20, 21], which is attributed to both extrinsic (such as physiological) and intrinsic (such as genomic and commensurate proteomic responses) conditions. This would therefore infer that there is a pressing need for development of an appropriate biosensor that would inform real-time disinfection of microbial pathogens post non-thermal treatment processes, such as PPGD. Previous researchers have revealed that the application of high voltage pulses to gas-injected test liquids (or PPGD) results in the formation of a plasma that causes free radicals such as dissolved ozone and hydrogen peroxide, free electrons, ultraviolet light (UV), acoustic shock waves and electric fields at levels between 10-50 kV/cm to be generated in the test liquids [10, 12]. It is likely that the combination of these biocidal properties contributed to the lethal action of PPGD during this study. Farrell et al. [22] reported that microbial pathogens, such as Candida albicans, are irreversibly inactivated by multi-hit biocidal processes where high intensity pulsed light caused death by cell membrane permeabilisation, necrosis, apoptois and nuclear damag. Pulsed electric discharge or PPGD has been shown to be effective for the oxidative destruction of structurally-related organic compounds such as dyes, phenol and aniline in aqueous solutions [13-16], yet there has been no published information on the ecotoxicity or safety of these plasma treated samples post treatment of priority substances. Ecotoxicology comprises that intergration of ecology and toxicology and its objective is to understand and predict effects of chemicals on natural communities under realistic exposure conditions [23]. This study also corrorborates the findings of Kelly et al. [24] who reported that the Microtox™ assay can be used in bench-scale wastewater treatment systms. While Velzoboer et al. [25] used the Mictox™ assay to show no appreciable ecotox effects at nomal concentrations of up to 100 mg/L for the nanoparticles TiO_2, ZrO_2, Al_2O_3 and CeO_2. This study highlighted the importance of ascertaining the ectoxicological status of treated priority

chemicals in aquaous environments, as despite reduction in concentration to less that 1ppm of phenol, the by-products products exhibited greater toxicity to that of untreated phenol. Therefore, there is a requirement to explore combinational mitigation approaches for addressing this problem. Ultimately, prioritized deployment of such mitigation decontamination technologies will be informed by risk assessment, managment (decision-making) and finances.

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REFERENCES

[1] Fernandez, M.P., Campbell, P.M., Ikonomou, M.G., Delvin, R.H. 2007. Assessment of environmental estrogens and the intersex/sex reversal capacity for Chinook salmon in primary and final municipal wastewater effluents. Environmental International, 33, 391-396.

[2] Tiedeken, E. J., Tahar, A., McHugh, B., Rowan, N. 2016. Monitoring, sources, receptors and control measures for three European Union watch list substances of emerging concern in receiving waters – a 20 year systematic review. Science of the Total Environment (574), 1140-1163). 46.

[3] Fent, K., Weston, A.A. & Caminada, D. 2006. Ecotoxicology of human pharmaceuticals. Aquatic Toxicology, 76(2), 122-159.

[4] Zhou, J.L., Zhang, Z. L., Banks, E., Grover, D. & Jiang, J. Q. 2009. Pharmaceutical residues in wastewater treatment works effluents and their impact on receiving river water. Journal of Hazardous Materials, 166, 655-661.

[5] Schwartz, T., Kohnen, W., Jansen, B., Obst, U. 2003. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water and drinking water biofilms. FEMS Microbial Ecology, 43 (3), 325-335.

[6] Heberer, T. 2002. Tracking persistent pharmaceutical residues from municipal sewage to drinking water. Journal of Hydrology, 266(3–4), 175-189.

[7] Nikolaou, A., Meric, S. & Fatta, D. 2007. Occurence patterns of pharmaceuticals in water and wastewater environments. Anal of Bioanal Chem, 387, 1225-1234.

[8] Zhang, Y. & Zhou, J.L. 2008. Occurrence and removal of endocrine disrupting chemicals in wastewater. Chemosphere, 73(5), 848-853.

[9] Verlicchi, P., Al Aukidy, M. & Zambello, E. 2012. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. Science of The Total Environment, 429(0), 123-155.

[10] Rowan NJ, S. Espie, J. Harrower, J. G. Anderson, L. Marsili, and S.J. MacGregor. 2007. Pulsed plasma gas inactivation of microbial pathogens in chilled poultry wash water. Journal of Food Protection. *Journal of Food Protection* **70** (12): 2805-2810.

[11] Rowan, NJ, S. Espie, J. Harrower, H.P., Farrell, J. G. Anderson and S. J. MacGregor. 2008. Evidence of lethal and sublethal injury in microbial pathogens exposed to pulsed plasma gas discharge treatments. Letters in Applied Microbiology 46 (1) 80-86.

[12] Hayes, J., Kirf, D., Garvey, M., Rowan, N. 2013. Disinfection and toxicological assessments of pulsed plasma gas discharge and pulsed UV light treated water containing the waterborne enteroparasite *Cryptosporidium parvum*. Journal of Microbiological Methods (94); 325-337.

[13] Willberg, D.M., Lang, P.S., Hochemer, R.H, Kratel, A., Hoffmann, M.R. 1996. Degradation of 4-chlorophenol, 3-4-dichloroaniline, and 2,4,6-trinitrotoluene in an electrohydraulic discharge reactor. Environmental Science and Technology, 30 (8), 2526-2534

[14] Bubnov, A.G., Grinevich, V.I., Kuvykin, N.A. 2004. Phenol degradation features in aqueous solutions upon dielectric-barrier discharge treatment, high energy. Chemistry 38 (5).

[15] Hao, X.L., Zhou, M.H., Lei, I.C. 2007. Pulsed discharge plasma induced Fenton-like reactions for the enhancement of the degradation of 4-chlorophenol in water. Chemosphere 66, 2185-2192.

[16] Amin, M.T., Ryu, S., Park, H. 2007. Degradation of phenol and bisphenol using a discharged water generating system. Journal Water Supply Research Technology Aqua 56 (3), 203-216.

[17] Yaqub, S., J. G. Anderson, S. J. MacGregor and N. J. Rowan. 2004. Use of fluorescent metabolic stain to assess lethal and sub-lethal injury in food-borne bacteria exposed to pulsed electric fields. Letters in Applied Microbiology 39: 246-251.

[18] Garvey, M., Hayes, J., Clifford, E., Rowan, N. 2015. Ecotoxicological assessment of pulsed ultraviolet light treated water containing microbial species and *Cryptosporidium parvum* using a microbiotest test battery. Water and Enviromental Journal, 29, 27-35.

[19] Kramer, B. & Muranyi, P. 2013. Effect of pulsed light on structural and physiological properties of *Listeria innocua* and *Escherichia coli*. Journal of Applied Microbiology, doi: 10.1111/jam 12394.

[20] Rowan, N., Valdramidis, V.P., Gomez-Lopez, V.M. 2016. A review of quantitative methods to describe efficacy of pulsed light inactivation data that embraces the occurrence of viable non culturable microorganisms. Trends in Food Science and Technology 44(1),79-92.

[21] Rowan, N. J. 2004. Viable but nonculturable forms of food and water-borne bacterial pathogens: quo vadis? Trends in Food Science and Technology 15: 462-467.

[22] Farrell, H.P., Hayes, J., Laffey, J.G., Rowan, N.J. 2011. Studies on the relationship between pulsed UV light and the simultaneous occurrence of molecular and cellular damage in clinically-relevant *Candida albicans*. Journal of Microbiological Methods 84: 317-326.

[23] Chapman, P.M. 2002. Integrating toxicology and ecology: putting the eco into ecotoxicology. Marine Pollution Bulletin, 44, 7-15.

[24] Kelly, C.J., Jumsaroj, N., Lajoie, C.A. 2004. Assessing wastewater metal toxicity with bacterial bioluminescence in a bench-scale wastewater treatement system. Water Research, 38, 423-431.

[25] Velzeboer, I., Hendriks, J., Ragas, M.J., Van de Meent, D. 2008. Nanomaterials in the environment: aquatic ecotoxicity of some nanonmaterials. Environmental Toxicological Chemistry. 27, 1942-1947.