**Toxicity assessments of the selected sulphonamides solutions after ozonation in comparison to oxidation by fungal peroxygenase**

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Veterinary drugs from the sulphonamides group (SAs), such as sulfamethazine (SMZ) and sulfadiazine (SDZ), are the oldest chemotherapeutic agents used for antimicrobial therapy and due to their bioactivity, they still play an important role. Hence, traces of these compounds, alone or in combination, have been repeatedly detected in the environment (De Liguoro, 2009).

Traditional methods (wastewater first and secondary treatment) show poor removal efficiency for these biologically active chemicals, thus additional treatment is required (O’Shea, 2004).

Very good results in the decomposition of these drugs in the aquatic environment were achieved by ozonation and using an oxidation process with unspecific peroxygenase from *Agrocybe aegerita* (*Aae*UPO) (Lemańska-Malinowska, 2015). Nonetheless, special attention should be paid to the risk of by-products’ formation, which may cause potential toxic effects on the living organisms (O’Shea, 2004).

Therefore, the aim of this study was to compare the acute toxicity of SMZ and SDZ before and after both processes in a range of trophic levels. For this purpose, toxicity tests on *Vibrio fischeri*, *Pseudokirchneriella subcapitatum (CCAP 278/4)* and *Daphnia magna* were conducted, as described below.

Toxicity tests on *Vibrio fischeri* bacteria were carried out using Microtox® analyser according to the Whole Effluent Toxicity (WET) test procedure. Acute Toxicity Reagent® was prepared according to the WET protocol every time before each test. The samples in the experimented were tested in five distinct concentrations from 100% to 6.25% of the overall original concentration. Tests were repeated in triplicate for each concentration and a blank control. The changes in the luminescence were measured after 15 minutes using Microtox Omni® software.

The effect of test solutions on the growth of the *Pseudokirchneriella subcapitatum (CCAP 278/4, green algae)* was measured as the rate of change of biomass. The biomass of the cultures was measured by chlorophyll extraction as an estimator of biomass at times 0, 24, 48 and 72 hours. Tests were carried out in continuous illumination, at 230C±20C. Jaworski’s Medium (JM) was used as a growth medium. Percent inhibition of growth was calculated as the % difference between the average growth rate of the controls and each treatment (concentration range 3.125-18.75% v/v) replicate using SigmaPlot® software.

*Daphnia* acute toxicity tests were performed according to the OECD guideline 202 as range finding and definitive tests. The acute tests were conducted using seven different concentrations (5-51% v/v) and a blank control. After exposure for 24 h and 48 h the immobilization rate of individuals in each container was calculated. The 24 h and 48 h EC50 (immobilization) values were calculated using SigmaPlot® software. Samples were diluted in 1:2 ratio in OECD M7 medium.

The results obtained from toxicity tests were compared. Solutions of SDZ and SMZ before ozonation show no significant changes in toxicity compared to the solution after the ozonation process was applied. No EC50 values could be calculated for the test with bacteria. SDZ and SMZ caused 50% of algae growth inhibition after 72 h of exposure at concentration 4.95% v/v and 6.54 % v/v respectively. EC50 values after 72 hours were 9.82% v/v and 9.83% v/v. For the freshwater crustacean the EC50 values were in the range 25.98- 33.55 %. It is concluded, that the ozonation process significantly decreased toxicity of the samples.

For the enzymatic oxidation of selected SAs results show a slight increase in toxicity for mixtures after the process in comparison with solutions before oxidation. Tests on *V. fischeri* showed a EC50 equal to 50.69 % for SDZ solution after this process was applied. SDZ and SMZ caused 50% of *P. subcapitatum* growth inhibition after 24 hours of exposure at concentration 5.14% v/v and 4.83 % v/v, respectively. After 72 h exposure the growth of algae was almost completely inhibited. EC50 values for *D. magna* test were in the range 14.54- 15.37 %. No significant changes after and before enzymatic oxidation have been found. In general, SMZ and SDZ mixtures before and after ozonation are much less toxic when compared to the enzymatic oxidation process.

References

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