Ecotoxicological analysis of cyanotoxins and antibiotics as a threat to environmental health and exploration of statistical methods for the analysis

長崎大学大学院工学研究科
Nguyen Tan Duc

Chapter 1. Introduction and objectives of doctoral research

In this chapter, I provide general information and the importance of my academic field and its connection with environmental pollution. Besides, I also declare the motivation of Ph.D. research.

Chapter 2. Reviews of investigated antibiotics and cyanotoxins

In this chapter, intensive reviews of targeted contaminants of antibiotics and cyanotoxins, such as effect mechanisms, environmental pathways, and occurrences in aquatic environments, are implemented with a huge meta-analysis.

Chapter 3. Ecotoxicology of cyanotoxins in cyanobacterial bloom biomass to *Daphnia magna*

Subchronic effects of the cyanobacterial crude extracts (CCEs) from microcystin-producing and microcystin-free cyanobacteria, with different microcystin concentrations (1, 10, and 50 $\mu g\ L^{-1}$) on *Daphnia magna* were investigated. Results showed that both microcystin-containing (MCCE) and microcystin-free (NCCE) crude extracts insignificantly reduced survival but strongly enhanced the reproduction and somatic growth of organisms. However, the degradation of eggs and neonates of the gravid females exposed to the CCEs was observed. Besides, the feeding rate of *D. magna* exposed to MCCE has been increased significantly whereas no change in the feeding rate was observed for NCCE-exposed *D. magna.*

Chapter 4. Ecotoxicology and statistical investigation of antibiotics to *Daphnia magna*

The chronic toxicity test for *D. magna* was performed during 42 days under exposure to ciprofloxacin (CFX) and ofloxacin (OFX) concentrations of 50, 500, and 5000 $\mu g\ L^{-1}$. All exposure conditions did not cause mortality for *D. magna*. CFX exposure at 500 $\mu g\ L^{-1}$ resulted in an earlier oogenesis date and an increase of the brood size in the second birth, but OFX did not show such a hormesis effect on *D. magna* maturity and fertility. The Poisson-based generalized linear mixed-effects model considering overdispersion of brood size revealed that the reduction

of brood size was statistically significant for the exposure of CFX and OFX at 5000 μg L^{-1}. The OFX exposure showed that the fertility-suppressed effects continued for a longer period compared to the CFX exposure. On the other hand, the production of dead eggs as offspring degradation in CFX exposure at 5000 μg L^{-1} was more significant than in OFX exposure at 5000 μg L^{-1}.

Chapter 5. Ecotoxicology of five antibiotics and their mixture to *Simocephalus vetulus*

Ecotoxicology of fluoroquinolones, tetracycline, and their mixture under 48-hour acute exposures to 0, 0.625, 1.25, 2.5, 5, 10, 20, and 40 mg L^{-1} was investigated for *S. vetulus* thoracic limb rate (TLR). Gatifloxacin caused mortality rates of 90 and 100% in the exposures of 20 and 40 mg L^{-1}. The hormesis effects on *S. vetulus* TLR were found in the exposures of 10, 20, and 40 mg ciprofloxacin L^{-1}. Also, ofloxacin was seen to induce the hormesis effects in the exposure of 1.25 mg L^{-1} onwards. The reductions of *S. vetulus* TLR were observed in the gatifloxacin test where organisms were exposed to 2.5, 5, and 10 mg L^{-1}. Delafloxacin and Tetracycline did not change *S. vetulus* TLR. Interestingly, antibiotic mixture only the suppressed *S. vetulus* TLR in the highest exposure of 40 mg L^{-1} (pooled concentration of five antibiotics).

Chapter 6. Statistical investigation of Bayesian analysis for ecotoxicological data

I implemented the subchronically ecotoxicological studies for ciprofloxacin (CFX) and ofloxacin (OFX) in the exposure of 100, 200, and 400 μg L^{-1} to *Moina macrocopa* fertility. The obtained dataset was analyzed by two approaches for comparing decision-making No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC). The results showed that for CFX, the estimated NOEC and LOEC were 100 μg L^{-1} and 200 μg L^{-1}, respectively by two approaches of the Bayesian methods while being 200 μg L^{-1} and 400 μg L^{-1} respectively by a Frequentist method. For OFX, the Bayesian method with 95% highest density interval estimated the NOEC and LOEC to be 200 μg L^{-1} and 400 μg L^{-1}, respectively while Bayes factor and Frequentist method were the same where LOEC was over of a maximum tested concentration resulting in undefined NOEC.

Chapter 7. Summaries of four studies in Ph.D. dissertation

To infer the entire research included in a Ph.D. dissertation, I give a study-to-study summary in which the important results of each study from chapter 3 to chapter 6 are indicated in detail.