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Response of the Lichen *Evernia prunastri* to Exposure of Antibiotic

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Abstract. Tetracycline antibiotics are one of the main groups of antibiotics widely used in medicine and a major environmental concern. However, the effects on non-target organisms are not well studied. Thalli of lichens artificially were exposed to high levels tetracycline to determine the impact on physiological parameters (integrity of cell membranes, photosynthetic efficiency, viability) and oxidative stress response (membrane lipid peroxidation). The results of the experiment showed that exposure did not affect the lichen membrane damage as indicated by unchanged values in conductivity. The potential photosystem II efficiency (F_V/F_M) was susceptible to the impact of antibiotics. The concentrations of TBARS were markedly increased with increasing concentration of tetracycline. The results of the study supplemented the knowledge on the effects of pollution on organisms, such as veterinary antibiotics, on lichens and provided a better understanding of the mechanisms of toxicity.
Keywords: tetracycline, lichen, membrane permeability, chlorophyll fluorescence, oxidative stress

1. Introduction

The use of medicines has increased significantly in recent years. At the same time, the use of antibacterial medicines, which are among the most commonly used medicines in the world, is also increasing. As a result, these substances continuously increase in the environment before they can break down naturally (Gao et al., 2012). Concerning antibiotic contamination has been found in recent research that have examined the prevalence and persistence of antibiotics (Yang et al., 2021).

Tetracycline antibiotics are broad-spectrum antibiotics with antibacterial properties. Tetracycline (TC) is used in human medicine, for veterinary purposes and in the agricultural sector as a food supplement. Due to its characteristics and great efficacy, tetracycline is one of the most commonly used antibiotics in aquaculture and veterinary care (Daghrir and Drogui, 2013). The main way antibiotics enter the environment is through urine and faeces (Bártíková et al., 2016).

The many ecosystem components are negatively impacted by the high concentrations of antibiotics in the environment (Polianciuc et al., 2020). The degree of phytotoxicity is influenced by the kind and dosage of antibiotics. Antibiotics prevent growth by interfering with plant germination and development, and (Tasho and Cho, 2016). Antibiotics have the potential to negatively impact both aquatic and terrestrial non-target organisms (Kuppusamy et al., 2018).

Determining the toxicity of antibiotics to non-target species is essential to understand the impact of antibiotics on ecosystems. Cryptogams, especially epiphytic lichens, are frequently employed in field biomonitoring and laboratory investigations to evaluate environmental quality (Sujetovienė, 2015). Due to their sensitive physiology, lichens are more exposed to environmental changes than other organisms. Because the effects of pharmaceuticals on lichens remain unclear, to test this we investigated the effects of tetracycline on the physiological status of non-target organism – lichen *Evernia prunastri*.

2. Material and methods

2.1. Lichen material

Thalli of the lichen *Evernia prunastri* (L.) Ach. were collected in a remote area located 15 km from Kaunas city center (Lithuania). After collection, samples were transferred to the laboratory and were cleaned from any extraneous materials. Prior to treatment, samples were left in a climatic chamber under constant light and moisture conditions.

2.2. Tetracycline treatments

Treatment solutions containing tetracycline (TC) at concentrations 50, 250 and 500 mg L^{-1} were prepared. These comparatively large concentrations were chosen in order to get a clear response and determine which parameters were the most sensitive. Lichen thalli were preserved at light and room temperature for 24 hours after being incubated by shaking for 1 hour in 50 mL of solutions. The treatment was repeated twice more, after 48 and 72 h. Control samples were treated in the same way, but samples were incubated in deionized water. All treatments and analyses were run on three replicates.

2.3. Physiological analyses

The viability of the sample was evaluated using the dehydrogenase activity revealed by the reduction of triphenyltetrazolium chloride to triphenylformazan (Bačkor and Fahselt, 2005). Lichen samples were incubated in a solution containing 0.6% TTC and 0.005% Triton X 100 in a 50 mM phosphate buffer (pH 6.8) for 20 hours in the dark. Following the removal of lichens, ethanol was used to extract water-insoluble formazan at a temperature of 65 °C. The absorbance was then measured at 492 nm after tubes had been centrifuged. Results were expressed as absorbance units g^{-1} (fresh weight).

The cell membrane integrity was measured after the soaking of lichen thalli in deionized water (50 mL) for 1 h using conductivity meter.

Photosynthetic efficiency as a general indicator of stress was measured with a Plant Efficiency Analyser (Handy PEA, Hansatech, Pentney, UK). Fluorescence was measured on wetted samples, applying a saturating flash of light of 2400 μmol s^{-1} m^{-2} for 1 s after thalli were dark adapted for 15 min. Five replicates were measured for each sample.

2.4. Statistical analyses

Significance of differences was checked using the HSD Tukey test for post hoc comparisons ($p < 0.05$). One-way ANOVA was applied to evaluate the significancy of treatment effects.

3. Results and discussion

3.1. Effects on lichen viability

Treatments with TC solutions induced a significant reduction of sample viability (Fig. 1). At TC concentrations above 250 mg L^{-1}, vitality decreased by more than 60% compared to the control. The tetracycline had significant negative effect on viability of mycobiont ($F = 36.63$, $p < 0.001$).

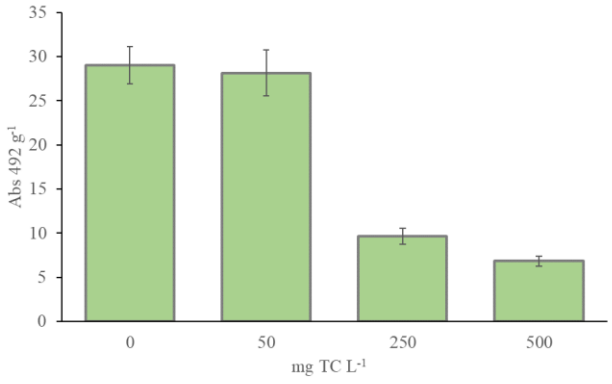

Figure 1. Effect of different concentrations of TC on viability of lichen *Evernia prunastri* thalli.

Photosynthetic efficiency was also sensitive and F_V/F_M values were significantly lower under the influence of TC compared the control indicating the significant negative effect of the tetracycline on the chlorophyll fluorescence parameter (Fig. 2, $F = 16.39$, $p < 0.0001$).

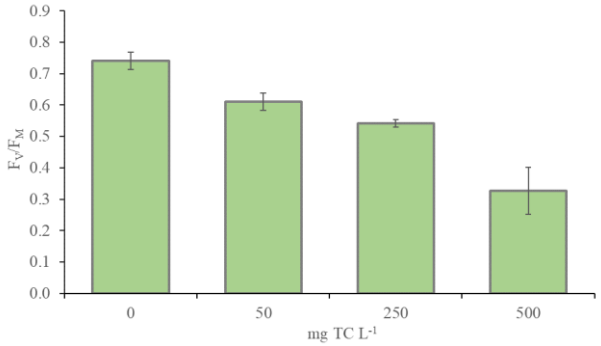

Figure 2. Effect of TC on the maximum photochemical efficiency of PSII (F_V/F_M) in *Evernia prunastri* thalli.

In our experiment the viability and photosynthetic efficiencey of *E. prunastri* was significantly reduced under the influence of high TC concentrations. The lichen Lobaria pulmonaria, in which the mycobiont and photobiont were damaged following exposure to the antibiotics ciprofloxacin and ampicilin, also underwent antibiotic treatment to confirm the stress response (Chavarria-Pizarro et al., 2022). Antibiotics' negative effects on the photobiont, green alga, included a reduction in photosynthesi. (Nie et al., 2013). This is consistent with research showing how TC affects algae and plants by interfering with metalolytic processes such water balance, photosynthesis, and respiration (Bártíková et al., 2016, Ve Van et al., 2023).

Treatments with TC did not induced damage to cell membranes because of relatively high variation in the values of rinsing water conductivity (data not shown). The significant increase in TBARS concentration in treated lichen thalli for 500 mg L^{-1} concentration was observed (Fig. 3). The remaining groups (50 and 250 mg L^{-1}) were slightly higher but did not differ from the control ($p < 0.05$). The overall effect of antibiotic TC on the induced oxidative stress in lichen was significant ($F = 5.14$, $p < 0.05$).

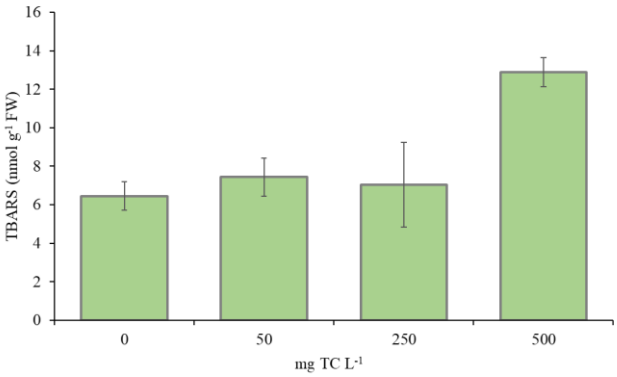

Figure 3. Effect of tetracycline on the thiobarbituric acid reactive substance (TBARS) concentrations in *Evernia prunastri* thalli.

Under the experimental conditions, the highest concentration of TC caused oxidative stressOur results show that the toxicity of antibiotics, including

tetracycline, to algae and bacteria reveals their sensitivity to these effects (González-Pleiter et al. 2013).

Conclusions

The artificial exposure of *E. prunastri* samples to the antibiotic tetracycline showed the relative effects of antibiotic treatments on mycobiont and photobiont viability. We did not find severe damage to the cell membrane. The study highlight the response of the symbiotic organism after the exposure to toxic compound.

References

Bačkor M. and Fahselt D. (2005), Tetrazolium reduction as an indicator of environmental stress in lichens and isolated bionts, *Environmental and Experimental Botany*, **53**, 125-133.

Chavarria-Pizarro T., Resl P., Kuhl-Nagel T., Janjic A., Fernandez Mendoza F., and Werth S. (2022), Antibiotic-induced treatments reveal stress-responsive gene expression in the endangered lichen *Lobaria pulmonaria*. *Journal of Fungi*, **8**, 625.

Daghrir R. and Drogui P. (2013), Tetracycline antibiotics in the environment: a review. *Environmental Chemistry Letters*, **11**, 209–227.

Gao P., Ding Y., Li H. and Xagoraraki I. (2012), Occurrence of pharmaceuticals in a municipal wastewater treatment plant: Mass balance and removal processes. *Chemosphere*, **88**, 17–24.

Polianciuc S.I., Gurzău A.E., Kiss B., Ștefan M.G. and Loghin F. (2020), Antibiotics in the environment: causes and consequences. *Medicine and Pharmacy Reports*, **93**, 231–240.

Sujetovienė G. (2015), Monitoring Lichen as Indicators of Atmospheric Quality. In: Upreti, D., Divakar, P., Shukla, V., Bajpai, R. (eds) Recent Advances in Lichenology. Springer, New Delhi.

Ve Van L., Quynh-Giao T., So-Ra K., Sang-Ah L., Hee-Mock O., Hee-Sik K. and Chi-Yong A. (2023), How do freshwater microalgae and cyanobacteria respond to antibiotics? *Critical Reviews in Biotechnology*, **43**, 191-211.

Nie, X., Wang, X., Chen, J., Zitko, V. and An, T. (2008), Response of the freshwater alga Chlorella vulgaris to trichloroisocyanuric acid and ciprofloxacin. Environmental Toxicology and Chemistry, 27: 168-173.

Bártíková H., Podlipná R. and Skálová L. (2016), Veterinary drugs in the environment and their toxicity to plants, *Chemosphere*, **144**, 2290-2301.

Tasho R. P. and Cho J. Y. (2016), Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: A review, *Science of The Total Environment*, **563–564**, 366-376.

Yang Q., Gao Y., Ke J., Show P. L., Ge Y., Liu Y., Guo R. and Chen J. (2021), Antibiotics: An overview on the environmental occurrence, toxicity, degradation, and removal methods, *Bioengineered*, **12**, 7376-7416.

Kuppusamy S., Kakarla D., Venkateswarlu K., Megharaj M., Yoon Y. E. and Lee Y. B. (2018), Veterinary antibiotics (VAs) contamination as a global agro-ecological issue: A critical view, *Agriculture, Ecosystems & Environment*, **257**, 47-59.

González-Pleiter M., Gonzalo S., Rodea-Palomares I., Leganés F., Rosal R., Boltes K., Marco E. and Fernández-Piñas, F. (2013), Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: Implications for environmental risk assessment, *Water Research*, **47**, 2050-2064.