Impact of microplastics and the insecticide Mospilan on survival, development, oxidative stress, and neurotoxic effects in *Bufotes viridis* tadpoles

Utjecaj mikroplastike i insekticida Mospilan na preživljavanje, razvoj, oksidativni stres i neurotoksične učinke kod punoglavaca vrste *Bufotes viridis*

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INTRODUCTION

The simultaneous presence of microplastics and pesticides in freshwater ecosystems represents a potential risk to aquatic organisms, particularly during the early developmental stages of amphibians. The green toad (*Bufotes viridis*) can serve as a suitable model organism for assessing the effects of pollutants on survival, development, and biochemical response.

The aim of this study was to evaluate the effects of two types of microplastics (PLA and PA11 blue MDT), individually and in combination with the insecticide Mospilan 20 SP (acetamiprid, 200 g/kg), on the survival, morphological development, and biochemical indicators related to oxidative stress and neurotoxicity in *Bufotes viridis* tadpoles.

MATERIALS AND METHODS

Bufotes viridis tadpoles were assigned to a control group and treatment groups exposed to the insecticide Mospilan 20 SP (acetamiprid, 200 g/kg), microplastics (PLA and PA11 blue MDT), and their combinations in FETAX medium. The exposure lasted 20 days (April 30 - May 19, 2026), with a total of 210 tadpoles included in the study, distributed across 30 glass jars (seven individuals per jar). Each treatment comprised three replicates, with an individual jar serving as the experimental unit.
Mospilan concentrations were 0.1 µg/L (C1), 0.25 µg/L (C2), 0.5 µg/L (C3), 1 µg/L (C4), and 10 µg/L (C5). PLA and PA11 blue MDT microplastics were applied at a concentration of 0.01 g/L, both individually and in combination with the insecticide Mospilan at a concentration of 0.5 µg/L (PLA + Mospilan and PA11 + Mospilan). Particle sizes were 0.05 - 0.30 mm for PLA and 0.05 - 0.10 mm for PA11 blue MDT. Total length (TL), snout-vent length (SVL), body mass, and Gosner developmental stage (GS) were determined at the beginning and end of the experiment. In homogenized tissue (S9 fraction), activities of AChE (Ellman et al., 1961), GST (Habig et al., 1974), and CAT (Claiborne, 1985) enzymes, as well as total protein concentration (Bradford, 1976), were determined spectrophotometrically.

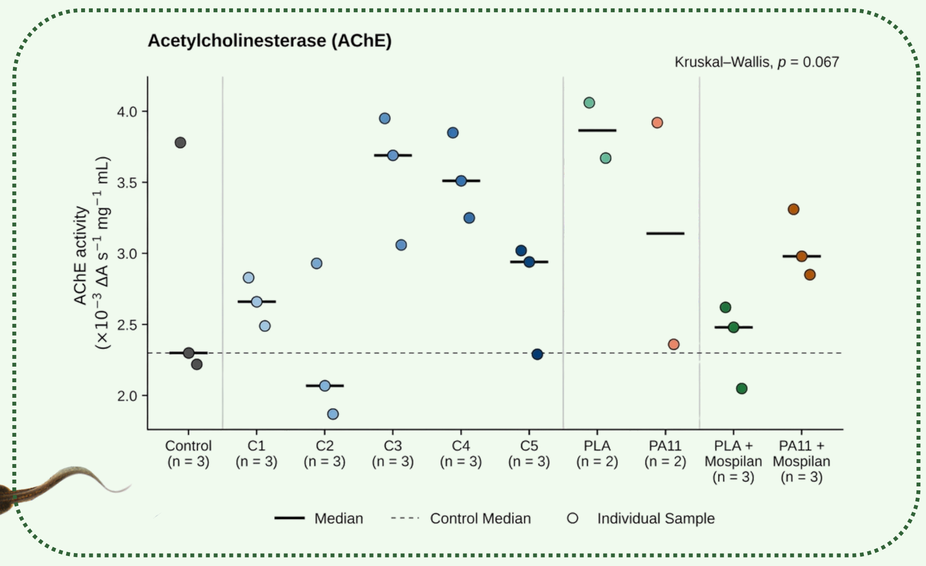


Figure 1. Acetylcholinesterase (AChE) activity depending on the applied treatment.

RESULTS

Preliminary results indicate the most pronounced effect on survival following exposure to PA11 blue MDT, with a survival rate of 9.5%, compared to 95.2% in the control group. Survival was 47.6% under PLA, 42.9% under PLA + Mospilan, and 57.1% under PA11 + Mospilan. In an overall comparison between groups exposed to Mospilan and microplastics, lower survival was observed in the microplastics group (p = 0.046), whereas a significant difference between groups was found for SVL (p = 0.018). No statistically significant differences were observed for TL, body mass, or Gosner stage. Protein-normalized AChE, GST, and CAT activities also showed no statistically significant differences, although GST exhibited the most pronounced variation between groups (p = 0.062), and AChE showed a borderline trend among individual treatments (p = 0.067).

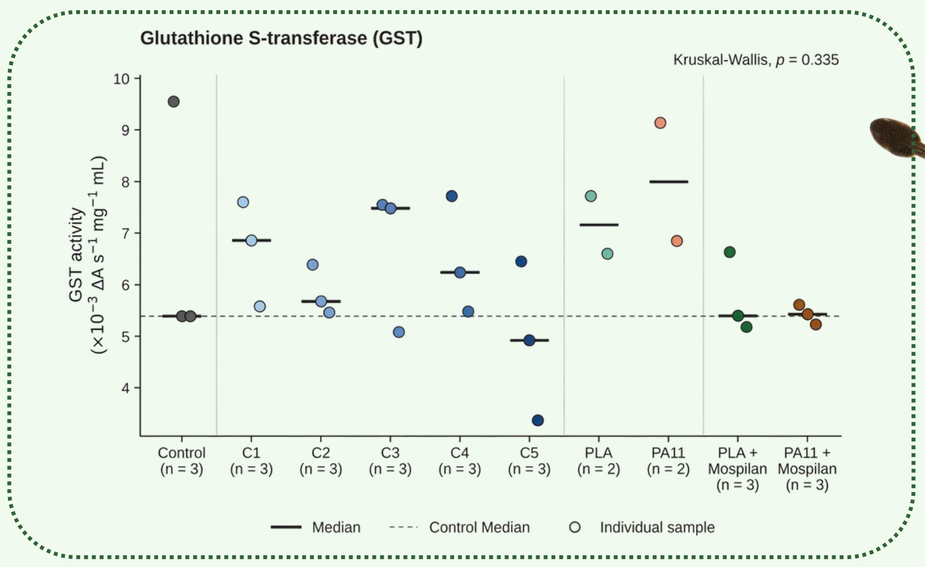


Figure 2. Glutathione S-transferase (GST) activity depending on the applied treatment.

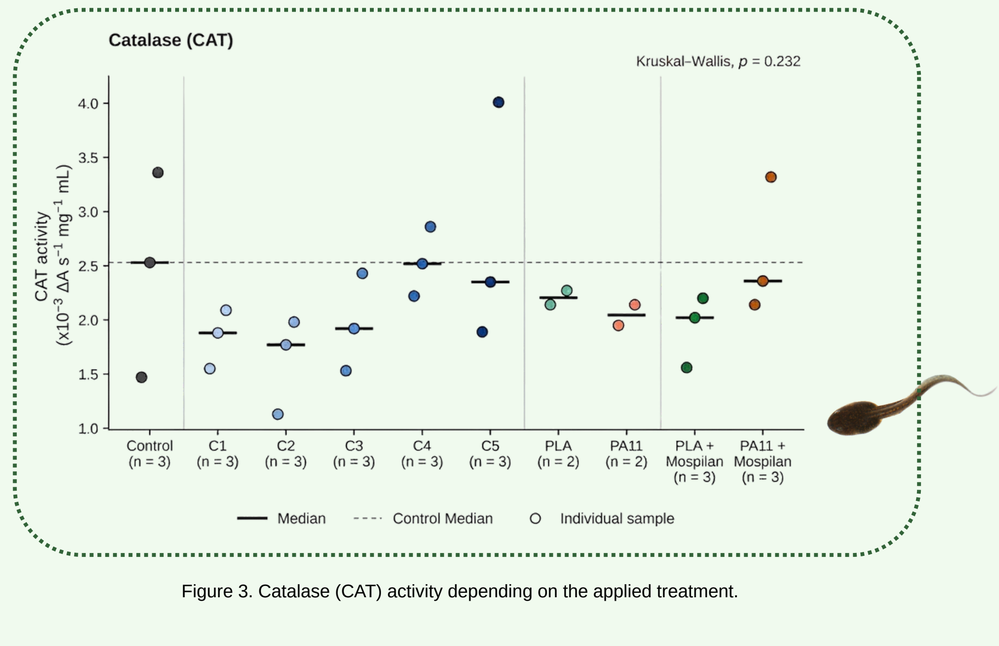


Figure 3. Catalase (CAT) activity depending on the applied treatment.

CONCLUSIONS

Preliminary results indicate a pronounced negative effect of certain treatments on tadpole survival, with the most marked impact observed for PA11 blue MDT microplastics. The observed differences in snout-vent length further suggest an influence of the treatments on tadpole development. Biochemical responses indicate complex effects of individual and combined exposure to microplastics and the pesticide; however, changes in AChE, CAT, and GST activities did not reach statistical significance. These results highlight the need for further research into neurotoxicity, oxidative stress, and the interactions between these pollutants in aquatic ecosystems.

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