Elevated temperature impact on toxicological effects of pesticides, antimicrobials and their mixtures to duckweed *Lemna minor*

MIŠKELYTĖ D. [1,2*], **, ŽALTAUSKAITĖ J.** [2], **MANUSADŽIANAS L.**[1]

[1]State Scientific Research Institute Nature Research Centre, Akademijos g. 2, 08412 Vilnius, Lithuania
[2]Vytautas Magnus University, Department of Environmental Sciences, Kaunas dstr., Lithuania

*corresponding author: e-mail: diana.miskleyte@vdu.lt

Abstract. Anthropogenic chemical pollution has the potential to pose one of the largest environmental threats to humanity, but global understanding of the issue remains fragmented. Water pollution becomes alarming since anthropogenic activity results in the contamination of water bodies with various contaminants, which in turn reduces ecosystem functioning and poses human health risks. Pesticides and pharmaceutical and personal care products (PPCPs) stand out for their widespread occurrence and highly diverse biological effects and are considered as emerging contaminants (ECs). Despite frequent detection in surface waters, the mixture effects of these emerging contaminants on the aquatic organisms remain unclear. In addition, there is a need to understand how changing environmental factors impact on joint effects of emerging contaminants as they often occur in the environment. To address the knowledge gap of the effects of emerging contaminants and their mixtures in a changing climate, we aimed to test the elevated temperature effect on mixed exposures of frequently detected pesticide Terbuthylazine (TBA) and antimicrobial Triclosan (TCS) to duckweed *Lemna minor*. Duckweeds *L.minor* were exposed to TBA and TCS individually and in mixtures (TCS+TBA) under different temperature regimes (23˚C and 27˚C). *L.minor* morphological indicators (frond area), biochemical indicators as well as oxidative stress damage were measured. Results showed elevated temperature exacerbated negative effects of terbuthylazine, triclosan and their mixture.

Keywords: Triclosan, terbuthylazine, climate change, Lemna minor

1. Introduction

Freshwater ecosystems constitute only 0.01 % of the water on Earth and less than one-tenth of the global land surface area but are the habitat of approximately 10 % of all recorded species including 30 % of all vertebrates (Suring, 2020). Due to anthropogenic activities there are many chemicals in our environment thus, many of these water systems are currently under ecological concern because of significant biodiversity losses (Beketov et al., 2013). Triclosan (TCS), a broad-spectrum antibacterial is extensively used in personal care products medical devices (Bedoux et al., 2012) and is one of the most frequently detected emerging contaminants in the environment. In the EU, herbicide terbuthylazine (TBA), a member of the chloro-s-triazine group, has emerged as one of the most frequently detected herbicides, replacing the banned atrazine (Di Guardo et al., 2020). The excessive use and disposal of TCS containing disinfectants and antiseptics raises concerns about the negative effects on human health and the environment (Mukherjee et al., 2021). In addition to the direct stress caused by chemical contamination, there are certain environmental stressors (such as temperature) that might cause additional stress. There is a growing body of evidence that increased temperature due to climate change will have a significant impact on the fate, distribution, and toxicity of environmental contaminants (Bhangare et al., 2022). As emerging contaminants are common in the environment, it is important to understand how changing environmental conditions affect the joint impacts of these contaminants. To our knowledge, climate change's effects on emerging pollutants mixture have not been tested yet.

2. Materials and methods

Experiments with *L.minor* were conducted in the controlled environment growth chambers. 6 different Triclosan (TCS) and Terbuthylazine (TBA) concentrations (range from 1 to 200 μg L^{-1}) were selected based on reported concentrations that were detected in aquatic environment and dose-finding pre-tests to obtain dose-response curve. TCS and TBA were exposed to *L.minor* in glass jars (450 ml), at 5 replicates in a 200 ml Steinberg growth medium (pH 5.5 ± 0.20). Single TCS and TBA as well as their mixture (TCS+TBA) toxicity experiments following 10 days exposure were conducted according to standardized OECD protocol No. 221 (OECD 2006) under two different temperatures: standard (23 ± 1 ºC) and elevated temperature (27 ± 1ºC). Mixture of TCS and TBA were formed based on effective concentration (EC_{50}) values from single chemical toxicity, where selected TU (toxic units) (0.25 – 2 TU) were used in a mixture. After 10 days of exposure *L.minor* growth parameters (total frond area (FA) were evaluated using ImageJ software. Dose-response analyses were performed using the morseDR R-package (R software, 4.4.1 version).

3. Results and discussion

As for single TBA and TCS effect, concentration of both tested chemicals had significant effect on *Lemna minor* total frond area (FA) under both tested temperature regimes (ANOVA, Table 1). Results showed that under both tested temperature regimes (23ºC ir 27ºC), TCS and TBA had a negative impact on *L.minor* growth. Strong negative correlation was detected (r= -0.73 – -0.84, $p < 0.05$) between concentration of chemicals and *L.minor* FA. However, elevated temperature (27 ºC) exacerbated the negative effect of the pollutants since the EC_{50} of both compounds was lower at elevated temperatures (Table 2). TCS was reported to inhibit algal growth, chlorophyll synthesis, and induce oxidative stress (Fekete-Kertész et al., 2018). Terbuthylazine toxicity to aquatic organisms is generally poorly documented. Some studies demonstrate a notable sensitivity of the microalgae *R. subcapitata* to terbuthylazine (Cedergreen and Streibig, 2005).

Table 1. Results of factorial ANOVA. Main factors are concentration (Conc) and temperature (Temp).

	TCS	TBA	Mixture
Conc	158.05***	479.83 ***	373.42 ***
Temp	-	6.44*	10.187 **
Conc x Temp	4.71***	20.87 ***	21.63 ***

*p < 0.02, ** p <0.01; *** p <0.001

A factorial analysis (ANOVA) revealed that temperature, chemical concentration, and their combined effect all had a significant effect on the mixture's toxicity ($p < 0.05$, Table 1). As with single exposure of TBA and TCS, it was discovered that with increasing contaminant concentration (toxic unit, TU), the growth of *L.minor* had tendency to decrease (r= -0.92 – -0.96, $p < 0.05$). EC_{50} of a mixture (TBA+TCS) under standard temperature (23ºC) was 37.34 µg L^{-1} (Table 2). Elevated temperature enhanced toxicity of a mixture (TBA+TCS) as observed EC_{50} under 27ºC regime was lower in comparison with 23 ºC, namely 26.69 µg L^{-1}. Combined effects of environmental pollutants may be additive, synergistic, or antagonistic. Recently, TBA was reported to be more toxic in a mixture with pharmaceuticals for *Aliivibrio fisch* (Gobolos et al., 2024). In our experiment it was found that mixture of chemicals under 23 ºC was sligltly antagosnistic as predicted EC_{50} of mixture was predicted to be 36.98 µg L^{-1}, and observed was slightly higher (37.34 µg L^{-1}).

Table 2. *50% effective concentration (EC_{50}) values for L.minor frond area under different temperatures (23ºC ir 27ºC) with their 95% credible intervals (Q2.5 % and Q97.5%).*

	Temp	EC_{50}	Q2.5	Q97.5
TBA	23	39.11	33.24	45.99
TBA	27	20.71	17.42	24.13
TCS	23	34.99	27.59	43.87
TCS	27	17.09	17.41	24.05
Mix	23	37.34	32.98	42.05
Mix	27	26.69	23.04	30.94

4. Conclusion

Single triclosan and terbuthylazine exposure as well as their mixture had a negative effect on duckweed *Lemna minor* growth under standard and elevated temperature. Elevated temperature exacerbated negative effects of terbuthylazine, triclosan and their mixture. The toxicity of chemicals in the mixture did not differ a lot, however it was slightly antagonistic.

5. Acknowledgements

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