

# Midgut trypsin and lipase activities, hemolymph protein and lipids levels with integrated biomarker response (IBR) in gypsy moth larvae from clean and polluted forest after chronic exposure to benzo[a]pyrene

Anja Grčić<sup>1</sup>, Larisa Ilijin<sup>1</sup>, Aleksandra Filipović<sup>1</sup>, Dragana Matic<sup>1</sup>, Marija Mrdaković<sup>1</sup>, Dajana Todorović<sup>1</sup>, Vesna Perić Mataruga<sup>1</sup>.

<sup>1</sup>Institute for biological research „Siniša Stanković“ National Institute of the Republic of Serbia, University of Belgrade, Despot Stefan Blv. 142, 11060 Belgrade Serbia

## INTRODUCTION

Intense anthropogenic influence led to a significant increase in pollution of the biosphere, in which polycyclic aromatic hydrocarbons, especially benzo[a]pyrene (B[a]P) made a major contribution. Wet and dry deposition gets atmospheric B[a]P on the vegetation, an important sink, and a crucial link for B[a]P bioaccumulation in animals<sup>1</sup>. The gypsy moth is a phytophagous polyphagous insect that inhabits wide forest areas. Due to its vast appetite, it can pile great amounts of pollutants making it a suitable model system for biomonitoring the adverse effects of B[a]P. The larval midgut is the central metabolic place where trypsin and lipases provide efficient digestion of protein and lipids-rich food, showing sensitivity to chemical pollutants<sup>2,3</sup>. Molecular parameters can be affected by physiological and environmental factors, so different adaptations of insects to the contaminants should be considered during the assessment of biomarker potential.

## OBJECTIVES

The aim was to investigate chronic effects of dietary treatment with B[a]P on midgut enzyme activities of trypsin and lipase, as well as the content of total proteins and lipids in hemolymph in gypsy moth larvae from two populations - one from an unpolluted oak forest and the other from a polluted oak forest. Furthermore, we used the method of Integrated Biomarker Response (IBR) to summarize responses of multiple molecular parameters across different tissues to estimate their sensitivity to B[a]P exposure in terms of population origin.

## METHOD

Gypsy moth egg masses were gathered in Đerdap National Park forest, free of industrial pollution (unpolluted population of larvae, UP), and Bor forest contaminated by various byproducts of the mining industry (polluted population of larvae, PP). From hatching until the sacrifice (third day of the 5<sup>th</sup> instar) larvae were fed with a diet containing 0 ng (UP 0 ng and PP 0 ng), 5 ng (UP 5 ng and PP 5 ng), or 50 ng (UP 50 ng and PP 50 ng) of B[a]P in 1 g of dry diet (DD).

Corresponding author: anja.gavrilovic@ibiss.bg.ac.rs

Spectrophotometric assays were used for the determination of specific enzyme activities of trypsin and lipase in the homogenates of the midgut, as well as for the evaluation of total proteins and lipids in the hemolymph<sup>4-8</sup>. Two-way ANOVA followed by Tukey's post-hoc test was used for statistical analyses (GraphPad Software, Inc., USA). Statistical significance was determined at ( $p$ )<0.05. Excel software (Microsoft, USA) was used to calculate IBR values and to generate star plots<sup>9</sup>.

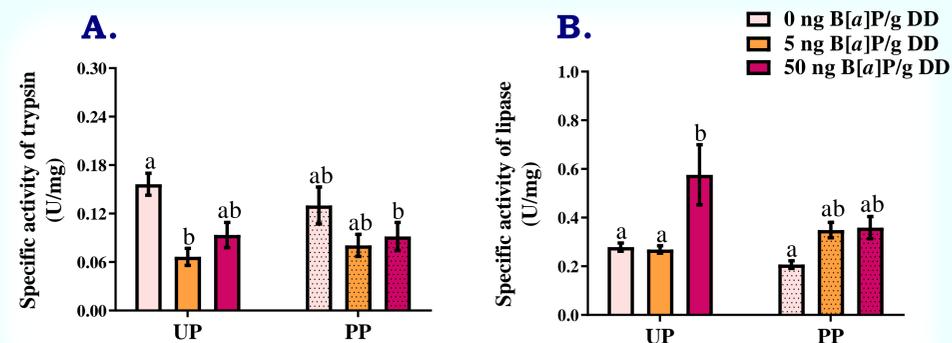


Figure 1. Trypsin (A) and lipase (B) specific activity in the gypsy moths midgut.

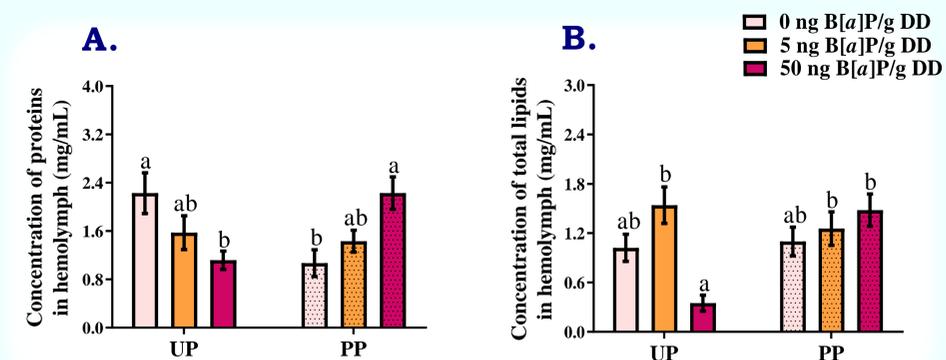


Figure 2. Total protein (A) and lipid (B) concentration in the gypsy moths hemolymph.

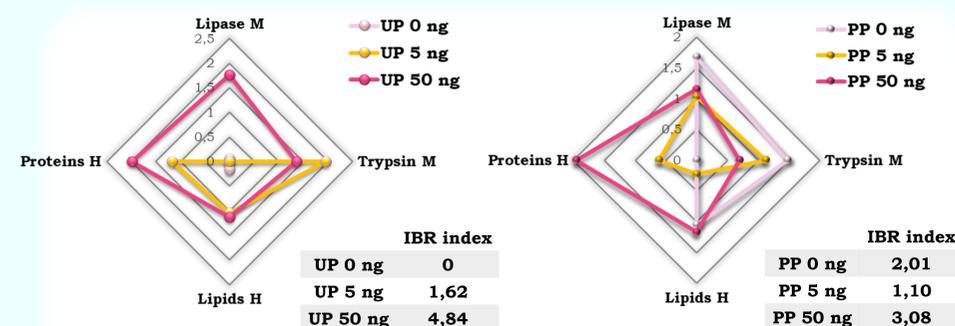


Figure 3. IBR index and star plots for gypsy moth larvae from UP and PP localities after chronic exposure to B[a]P.

## RESULTS

The specific activity of trypsin has significantly inhibited after the treatment with lower B[a]P concentration in UP ( $F=9.412$ ,  $p=0.0004$ ) (Fig.1.A), while a higher concentration of B[a]P significantly induced lipase activity in the same population of larvae ( $F=8.382$ ,  $p=0.0007$ ) (Fig.1.B). These enzymes showed no statistically significant changes in the PP. Hemolymph protein content was significantly affected by the chronic dietary exposure to the higher concentration of B[a]P in both populations of larvae, showing a decrease in the UP, and the elevation in the PP ( $F=10.16$ ,  $p=0.0002$ ) (Fig.2.A). Lipid concentration was not significantly changed under the B[a]P influence regarding the control groups (UP/PP 0 ng) but there was a meaningful difference between B[a]P treated larvae among two populations ( $F=7.16$ ,  $p=0.019$ ) (Fig.2.B). IBR index increased in a concentration-dependent way only in UP after the chronic exposure to B[a]P and the values were higher than the corresponding ones in the PP (Fig.3).

## CONCLUSIONS

Gypsy moth population from the unpolluted forest showed higher sensitivity to the chronic dietary exposure to B[a]P comparing to the population from the polluted forest, especially in terms of trypsin and lipase activity. Hemolymph protein level expressed well correspondence to B[a]P concentration in both populations but with inverse trends. The selected set of Gypsy moth larvae molecular parameters possess a good potential for B[a]P biomonitoring in the populations from unpolluted forest ecosystems.

## LITERATURE

- Wang et al., 2008. Environ Pollut. 151, 158-164.
- Araújo et al., 2008. Bull Entomol Res. 98, 417-424.
- Vlahović et al., 2015. Water Air Soil Pollut. 226, 1-13.
- Erlanger et al., 1961. Arch Biochem Biophys. 95, 271-278.
- Valaitis., 1995. Insect Biochem Mol Biol. 25, 139-149.
- Arreguín-Espinosa et al., 2000. Biotechnol Appl Biochem. 31, 239-244.
- Zöllner and Kirsch., 1962. Z Ges Exp Med. 135, 545-561.
- Bradford., 1976. Anal Biochem. 72, 248-254.
- Beliaeff and Burgeot., 2002. Environ Toxicol Chem. 21, 1316-1322.

This study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract No. 451-03-9/2021-14/200007.