

# Effects of chronic dietary cadmium on midgut superoxide dismutase (SOD) and catalase (CAT) in larvae from two *Lymantria dispar* populations

Dragana Matić, Milena Vlahović, Larisa Ilijin, Anja Grčić, Aleksandra Filipović, Marija Mrdaković, Vesna Perić-Mataruga

Department of Insect Physiology and Biochemistry, Institute for Biological Research “Siniša Stanković” - National Institute of Republic of Serbia, University of Belgrade, Despot Stefan Blvd. 142, 11060 Belgrade, Serbia

## 1. Introduction

Cadmium (Cd) levels in the environment have increased during decades of intensive industrial development and urbanization. *Lymantria dispar* has proved to be a suitable organism indicator to monitor Cd pollution in the forest ecosystems. Since insects accumulate heavy metals predominantly in the gut, it is not surprising that several enzymes in the midgut of *L. dispar* larvae, including antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), have been marked as promising biomarkers of Cd presence. Namely, Cd indirectly induces oxidative stress in the cell. Ideally, a suitable biomarker of exposure should respond to a toxicant consistently in different populations, regardless of their previous exposure to pollution. However, long-term exposure of the population to pollution often results in increased tolerance, and might affect sensitivity of biomarkers.

## 2. Objectives

- We aimed to determine specific enzyme activities and isoform patterns of SOD and CAT in the midgut of *L. dispar* larvae after chronic Cd treatment.
- To assess these parameters as biomarkers of Cd presence, we compared how two *L. dispar* populations with different histories of an exposure to pollution responded to dietary Cd.

## 3. Materials and methods

- Egg-masses of *L. dispar* originated from two populations inhabiting unpolluted and polluted localities in Serbia:

Unpolluted locality (UP)  
Kosmaj Mountain

Polluted locality (PP)  
near Ibar highway
- Larvae were fed wheat germ diet containing 0, 50 or 100 µg Cd/g dry food (C, Cd1, Cd2, respectively) starting from hatching until they were killed on the 3<sup>rd</sup> day of the 4<sup>th</sup> instar
- Experimental groups:

UP C

UP Cd1

UP Cd2

PP C

PP Cd1

PP Cd2
- Specific activities of SOD and CAT in the midgut homogenates were determined by spectrophotometric assays<sup>1,2</sup>.
- After SOD and CAT isoforms were separated by native PAGE<sup>3,4</sup>, the bands were quantified with ImageJ software.
- Enzyme activities were analyzed by one-way ANOVA followed by Tukey’s post-hoc test using GraphPad Prism 7 (GraphPad Software, Inc., USA). The level of statistical significance was  $p < 0.05$ .

## 5. Conclusions

- ✓ Higher SOD activity in control larvae originating from the site near the highway compared to those from the uncontaminated forest probably indicated the presence of traffic-related pollution that caused oxidative stress.
- ✓ Neither SOD nor CAT showed activation in response to Cd treatment.
- ✓ A decrease in SOD and CAT activity in both Cd-treated groups in the population from the polluted site could have been a result of the trade-off in favor of the other more efficient defense mechanism(s). Such trade-off might have led to the diminished expression of isoforms SOD-2 and SOD-3.
- ✓ Thus, a decrease in SOD and CAT activities after Cd exposure could be seen as an adaptive strategy of *L. dispar* population living in the chronically polluted habitat.
- ✓ Specific activities of SOD and CAT with SOD isoform pattern could be used as biomarkers of Cd exposure in contaminated environments.

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E-mail: dragana.matic@ibiss.bg.ac.rs

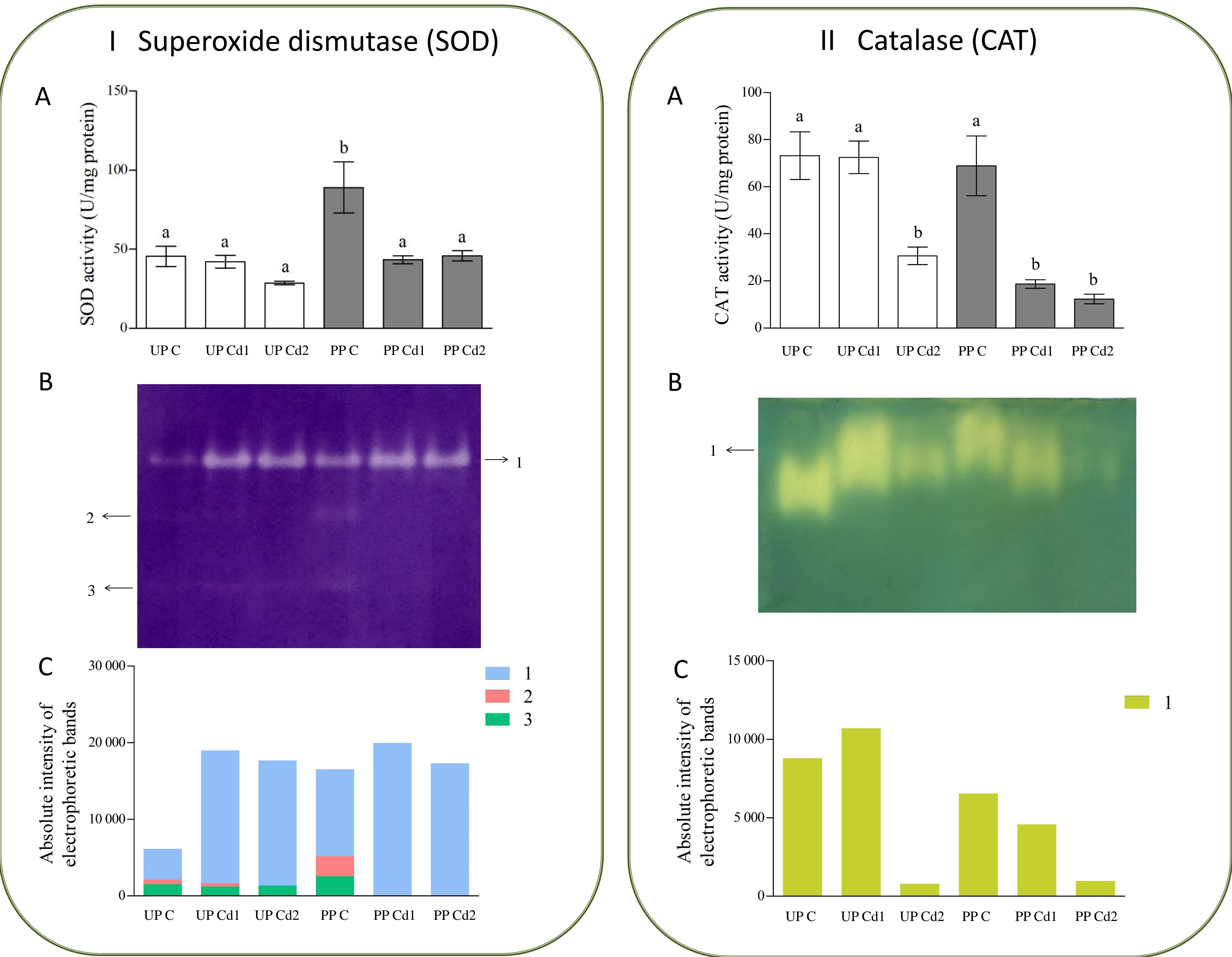
## 4. Results

### Specific activity and isoforms of SOD

- Specific activity of SOD was higher in control larvae from the polluted locality compared to the control group from the uncontaminated forest (I-A).
- An exposure to both Cd concentrations decreased SOD activity in larvae from the polluted site (I-A).
- Three SOD isoforms were detected in control groups from both populations. While isoform SOD-2 was absent in the population from Kosmaj after the treatment with higher Cd concentration, both SOD-2 and SOD-3 disappeared in all Cd-treated larvae from the site near the highway (I-B, I-C).

### Specific activity and isoforms of CAT

- In the population from the unpolluted locality specific activity of CAT was reduced at 100 µg Cd/g dry food (II-A).
- A decrease in enzyme activity was noticed at both Cd concentrations in larvae from the polluted site (II-A).
- The same pattern of Cd influence was observed for CAT isoform activity. Only one CAT isoform was present in both control and experimental larvae from both populations (II-B, II-C).



Effects of cadmium on superoxide dismutase (SOD) (I) and catalase (CAT) (II). Enzyme specific activities (A), native PAGE (B), and densitometric analysis of the bands (C) in the midgut of *L. dispar* larvae following chronic cadmium treatment. UP and PP – populations from the unpolluted and polluted sites, respectively; C - control, Cd1 and Cd2 - 50 and 100 µg Cd/g dry food, respectively. The bars show means±SE. Different letters denote significant differences among the experimental groups (Tukey’s post-hoc test,  $p < 0.05$ ). The results shown in panels B and C were obtained from a pooled sample.