

# Biomarkers of effect show spatial variation in plasma of white stork (*Ciconia ciconia*) nestlings from Croatia

Biomarkeri učinka u plazmi ptica bijele rode (*Ciconia ciconia*) s različitih lokaliteta u kontinentalnoj Hrvatskoj

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## Introduction

Enzyme activities are extensively used biomarkers to assess the response of organisms to various chemicals from the environment (Gonçalves et al. 2021). Biomarkers of effect could be used for providing crucial information and identifying harmful effects on non-target avian organisms such as white stork (*Ciconia ciconia*). Changes in acetylcholinesterase activity has been used as a diagnostic biomarker for pesticide exposure, such as organophosphate derivate and carbamates (Bang et al. 2019), and variation in carboxylesterase activity can be inhibited by organophosphate derivate, carbamates, diones, sulphonamides, etc. (Wheelock et al. 2008). White stork is a suitable species for biomonitoring due to being apex predators that forage in terrestrial and freshwater ecosystems. Since white stork is an altricial species, the nestlings are suitable bioindicators of environmental pollution by utilizing non-destructive methods and analysing biomarkers in blood (Bjedov et al. 2021).

## Material & methods

Information regarding potential exposure to harmful substances could be obtained from enzyme analysis. For this purpose, we compared biomarker response from seven locations in continental Croatia (Fig. 1): **Jakuševac** ( $n = 8$ ), **Lonjsko polje** ( $n = 12$ ), **Crnac polje** ( $n = 18$ ), **Jelas polje** ( $n = 11$ ), **Slavonski Brod - east** ( $n = 10$ ), **Podunavlje** ( $n = 30$ ) and **Donje Podravlje** ( $n = 17$ ). During **breeding season 2021**, 4 mL of white stork nestlings blood was sampled from brachial vein and plasma was obtained by centrifugation. Specific activities of biomarkers of effect: acetylcholinesterase (**AChE**) and carboxylesterase (**CES**) were analysed in nestlings' plasma (Bjedov et al. 2021). Linear mixed modeling was performed using the *lme* function. Using *post hoc* test *emmeans* we tested for significant differences between the locations. Levels of statistical significance was 0.05 ( $p$  value).

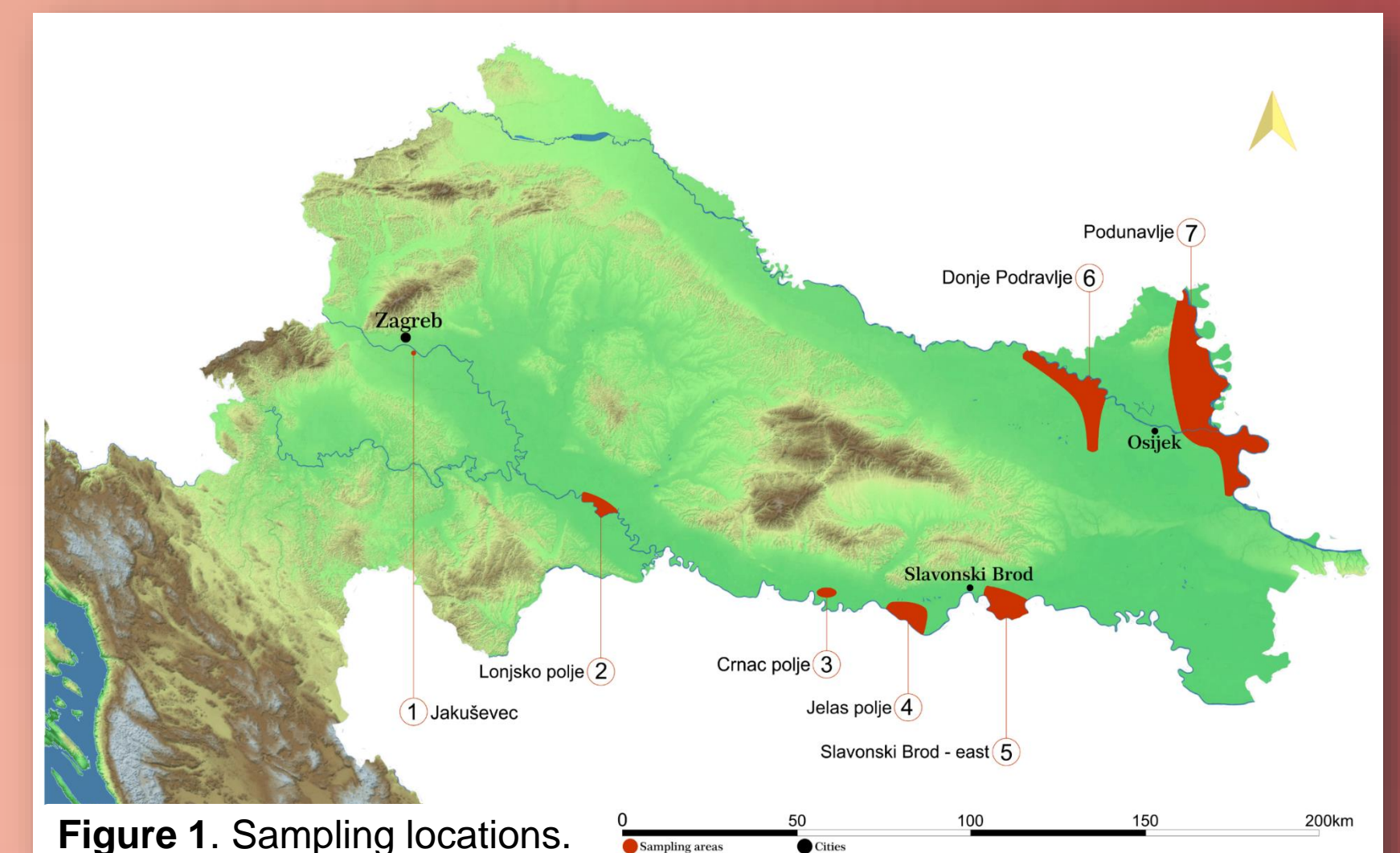


Figure 1. Sampling locations.



## Results

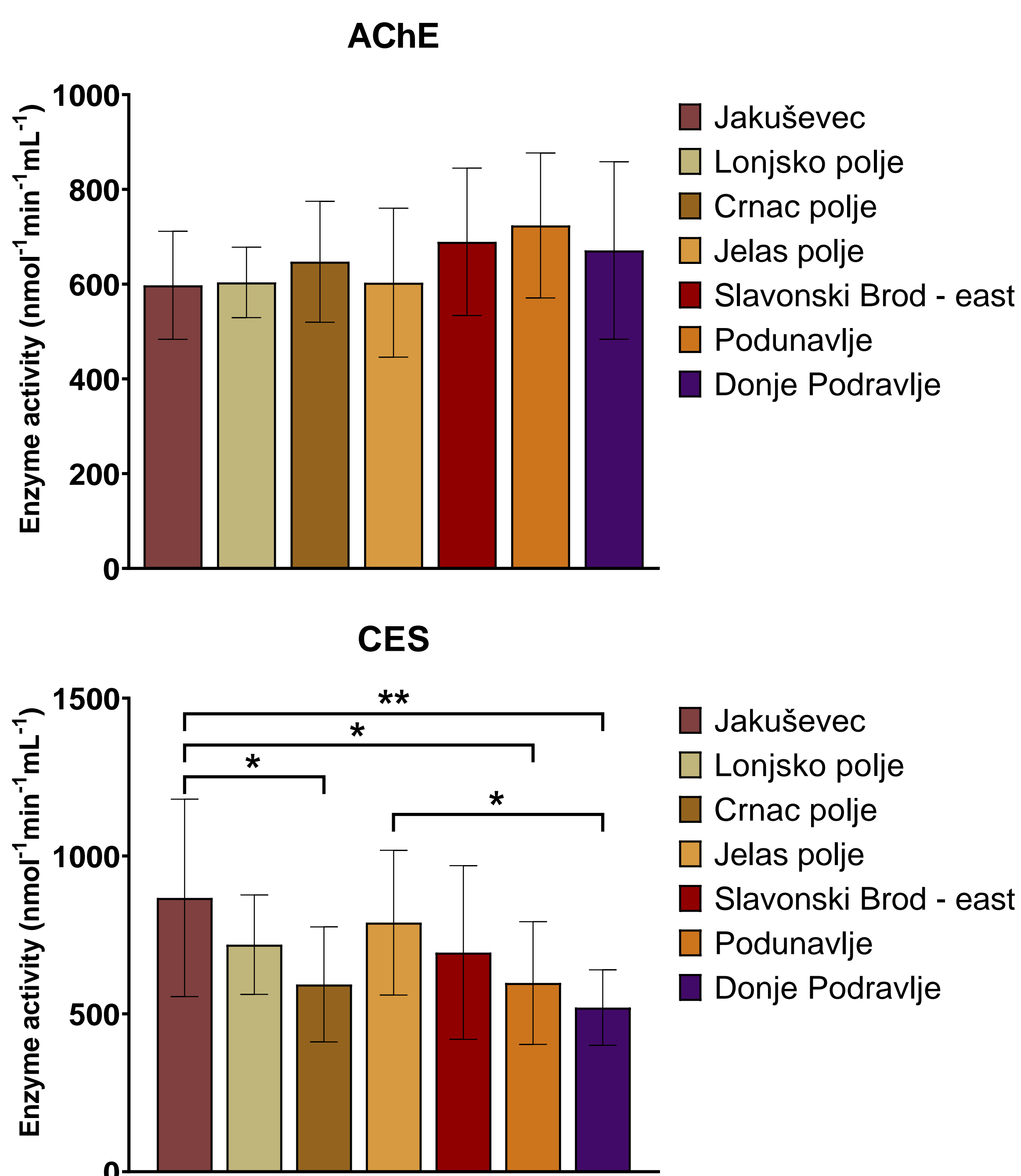


Figure 2. Specific acetylcholinesterase (AChE) and carboxylesterase (CES) activities in the plasma of white stork nestlings from different areas in Croatia. Statistical differences are indicated with \* ( $p < 0.05$ ) and \*\* ( $p < 0.01$ ).

## Conclusions

- ▶ No differences were found between the locations in AChE activity (Fig. 2).
- ▶ The lowest AChE activity was recorded in Jelas polje (Fig. 2).
- ▶ Inhibition in CES activity was recorded at Crnac polje, Podunavlje, and Donje Podravlje compared to Jakuševac (Fig. 2).
- ▶ **Results suggest presence of inhibitory pollutants** → areas are known for agricultural intensification, farming and excessive illegal hunting.
- ▶ Changes in CES activity indicate activation of detoxification mechanisms and shielding AChE from inhibitory xenobiotics.
- ▶ **White stork nestlings appear to be exposed to environmental toxicants.** However, a detailed chemical analysis of contaminants are needed for a complete comprehension of nestling's exposure and health status.

## References

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