Monitoring the effects of mixtures of chemicals in ecologically relevant species

Silvia Casini, M. Cristina Fossi, Tommaso Campani, Ilaria Caliani

Department of Physical, Earth and Environmental Sciences, University of Siena, Italy

Round Table: Ecosystem health and chemical mixture risk assessment and management

Control pests in agriculture and non target – ecologically relevant species
Some considerations ..

Mixtures, interactions … contaminants other than pesticides

Lack of data on the toxicological (sublethal) effects of single compounds and mixture

Lack of clear evidence of the actual exposure for the different species and populations.

Pollinator insects and pesticides: Apis mellifera

Ecologically relevant

keystone pollinator of wild plant species and agricultural crops

Economically relevant

From an economic point of view, the benefits have been estimated by the American Natural Resources Defense Council (NRDC) as over $ 15 billion per year for bee-pollinated crops and about $ 150 million per year for honey production.

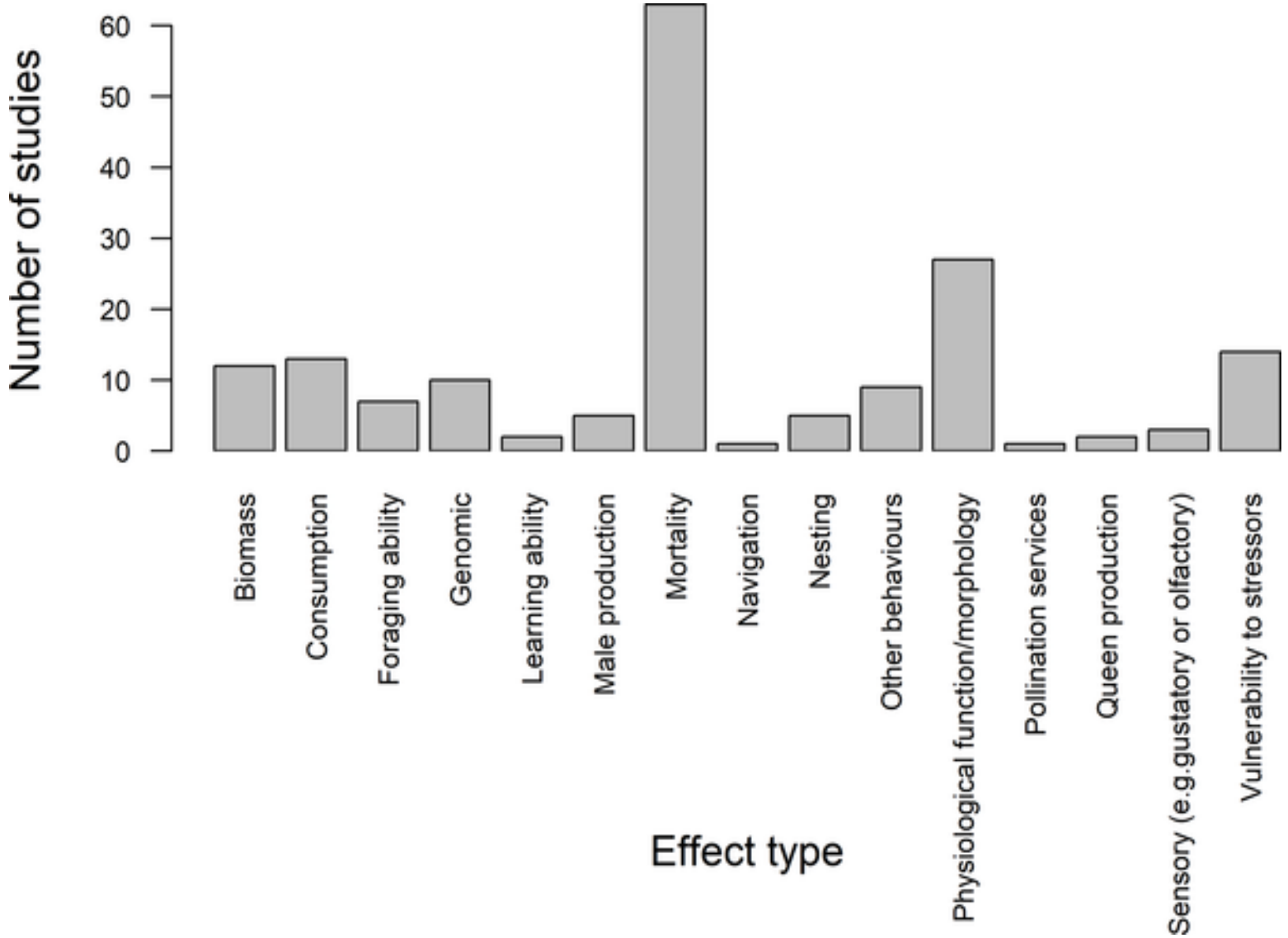
Globally endangered

parasites and diseases,
climate change
habitat loss,
genetic constraints,
beekeeper management issues,
widespread use of pesticides.

Exposed directly via **contact** during or after application, or **orally** through contaminated nectar and pollen

In a recent study by Prado et al. (2019)
half of contaminants found in pollen **(48%) are fungicides,**
30% are insecticides/acaricides and
13% are herbicides.

Cullen MG, Thompson LJ, Carolan JC, Stout JC, Stanley DA (2019) Fungicides, herbicides and bees: A systematic review of existing research and methods. PLOS ONE 14(12): e0225743. https://doi.org/10.1371/journal.pone.0225743



Sublethal effects ?

Effects at molecular, biochemical and cellular level

Early warning signals…

Aims of our work

To develop and apply a multi-biomarker approach and an Integrated Biomarker Response Index to assess the toxicological health status of honeybees (sub lethal effects)

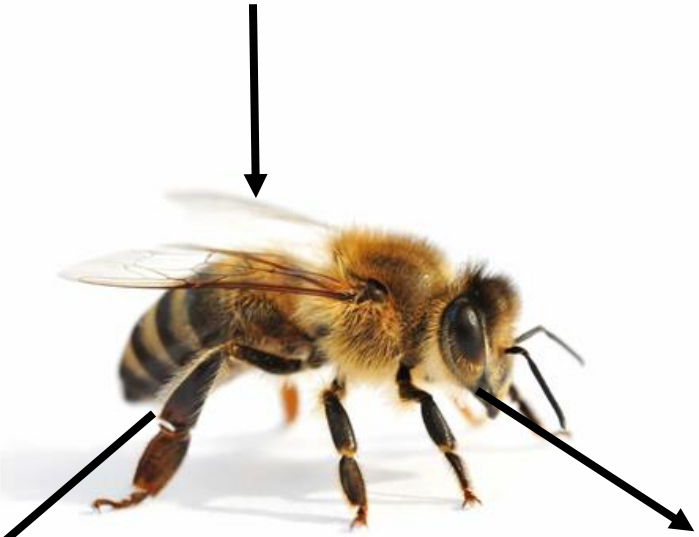
1) Investigate the toxicological effects of contaminants and mixtures (e.g. a commercial fungicide)

2) Monitoring of field populations of honeybees

Use of a set of biomarkers able to investigate alterations at different levels

Genotoxicity: Nuclear Abnormalities (NA)

Immunotoxicity: differential hemocytes count (DHC)



Immunotoxicity: Lysozime (LYS)

Biotransformation and metabolic processes:

Glutathione-S-transferase (GST)

Alkaline phosphatase (ALP)

Neurotoxicity

Acetylcholinesterase (AChE)

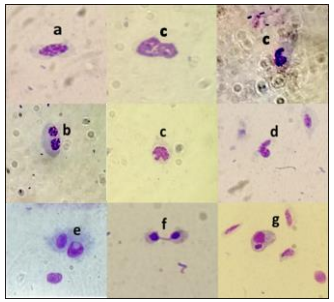
Carboxylesterase (CAE)

Biomarkers

Hemolymph

NA assay (Pacheco & Santos, 1997)

Differential Haemocytes Count (DHC) (Sapcaliu et al., 2009)

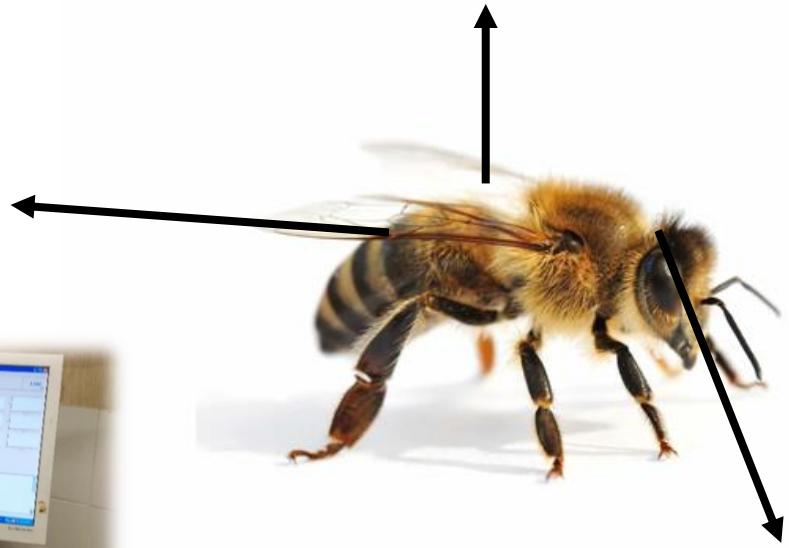


Midgut

GST (Habig et al., 1974, modified)

ALP (Bounias et al. 1996, modified)

LYS (Keller et al., 2005, modified)

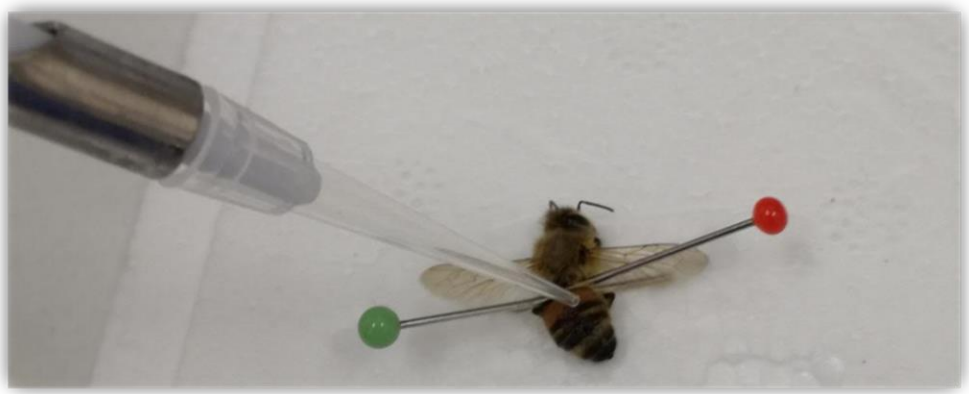


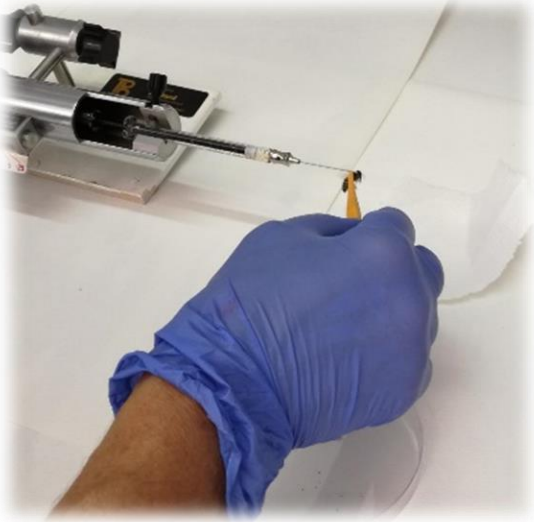
Head

CaE (Gomori, 1959, modified)

AChE (Ellman et al., 1961, modified)

LABORATORY Investigating the toxicological effects of a commercial fungicide

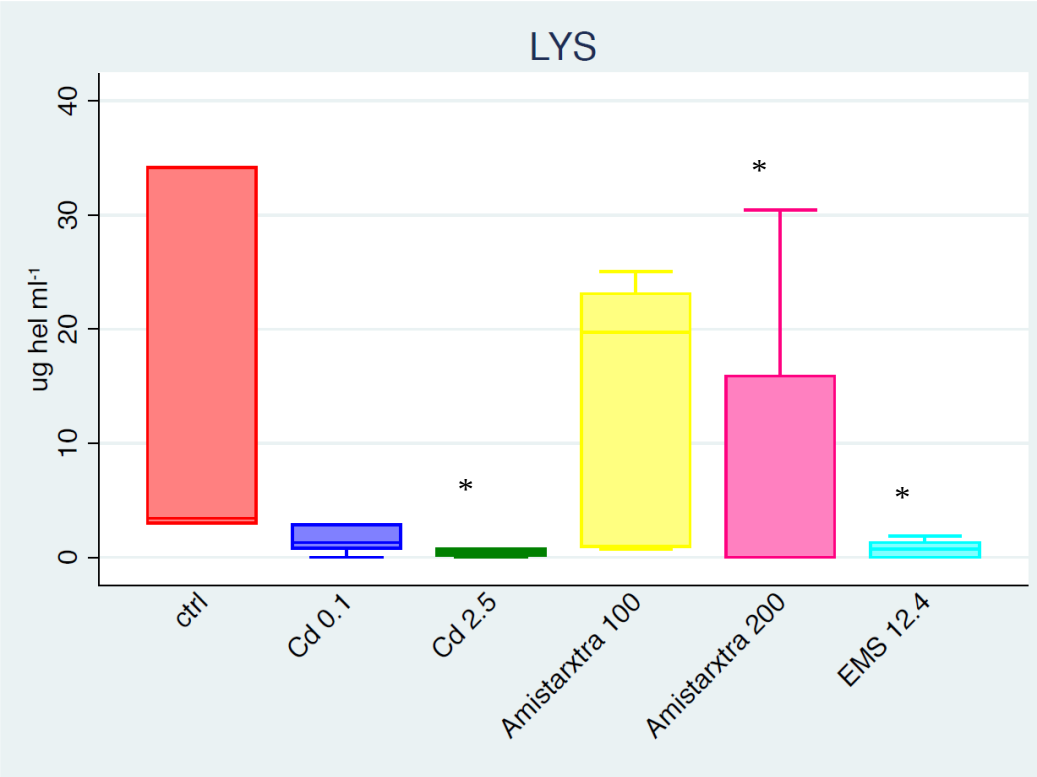




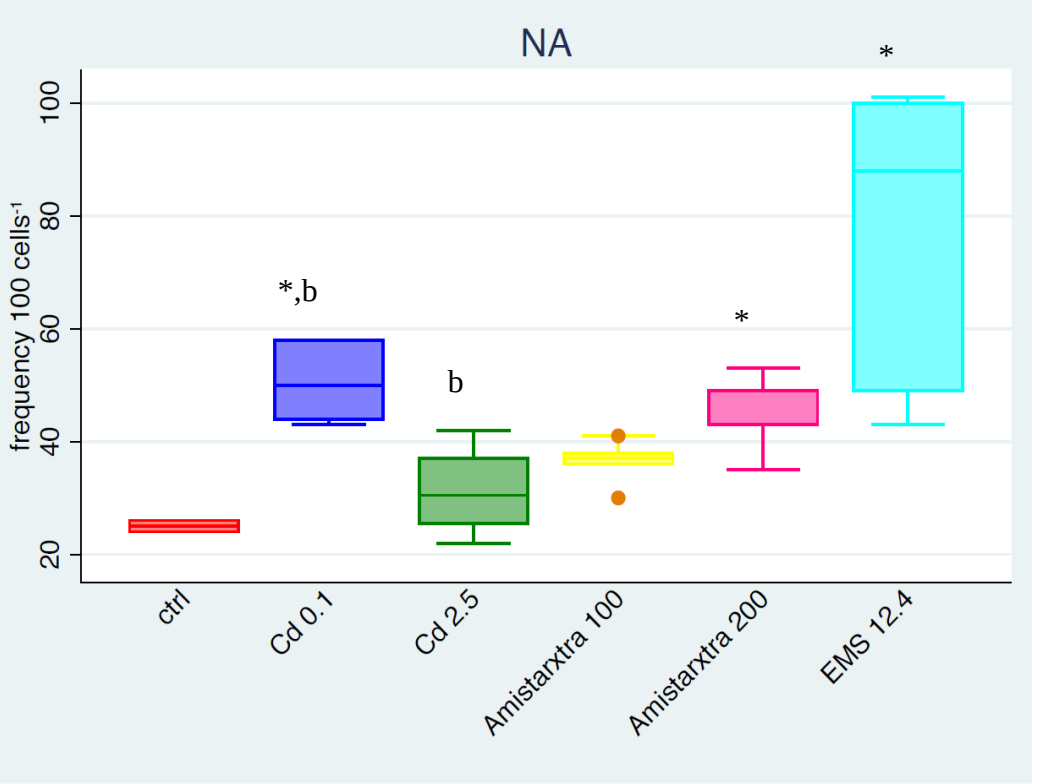
- **Cadmium:** 100 ppm e 2500 ppm
- **Commercial Fungicide - Amistar® Xtra** (azoxistrobin 18.2%, ciproconazole 7.3% + adjuvants)**:** 200g/L and 100 g/L field usage doses
- **EMS:** 0.1 M
- **Vehicle:** acetone

- 50 individuals - contact
- Time of exposure: 5 days

Biomarker of immunotoxicity



Biomarker of genotoxicity



* indicates statistical difference in comparison with controls ($p<0.05$)

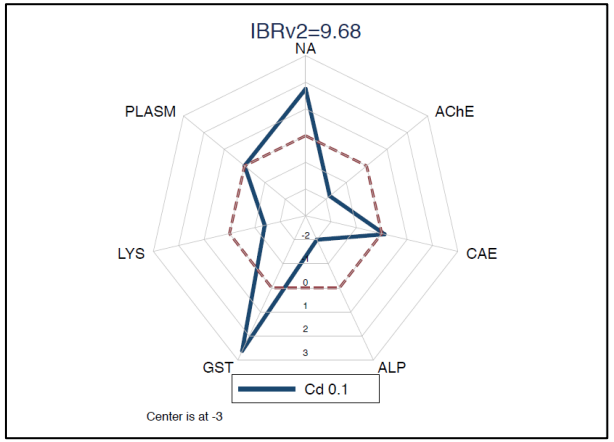
Amistar®xtra

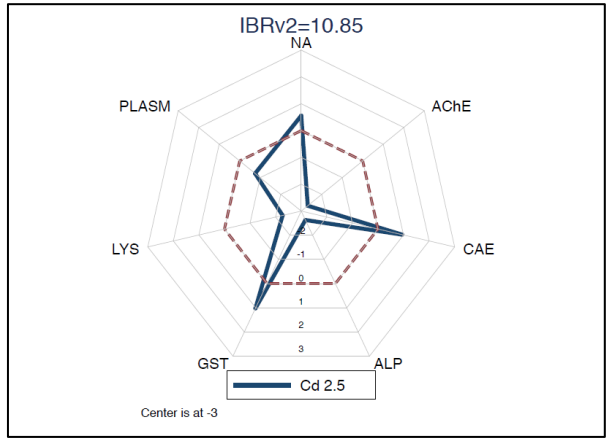
- Genotoxicity (NA assay)
- Neurotoxicity (Inhibition of esterases)
- Alteration of some immune system endpoints (decrease of lysozyme activity)
- Alteration of phase II biotransformation enzymes

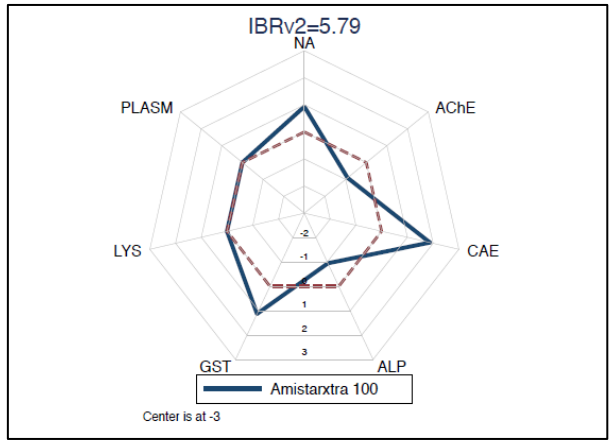
Cadmium

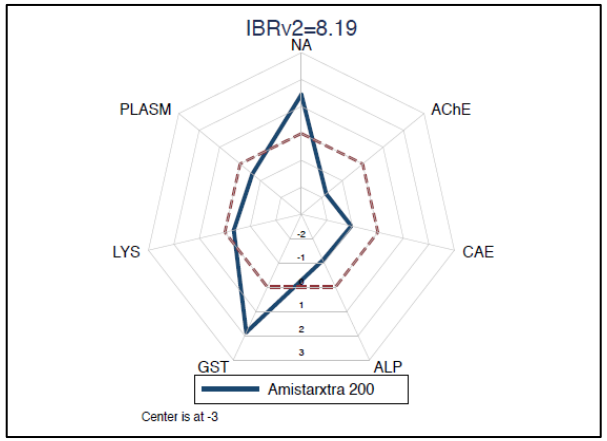
- Genotoxicity
- Neurotoxicity
- Alteration of phase II biotransformation enzymes and metabolic processes
- Alteration of some immune system endpoints

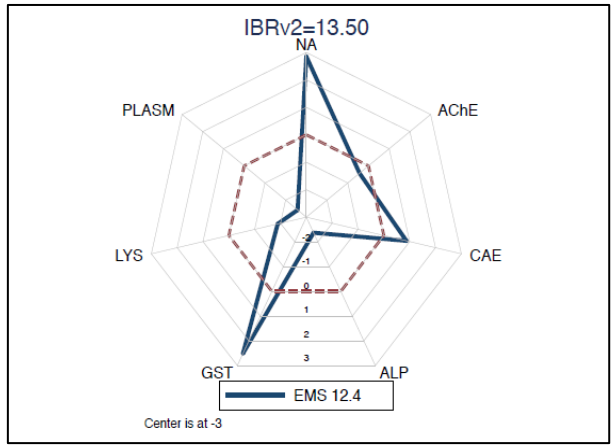
Spider graphs of the **Integrated Biological Response (IBRv2) Index** for each treatment.











FIELD STUDY

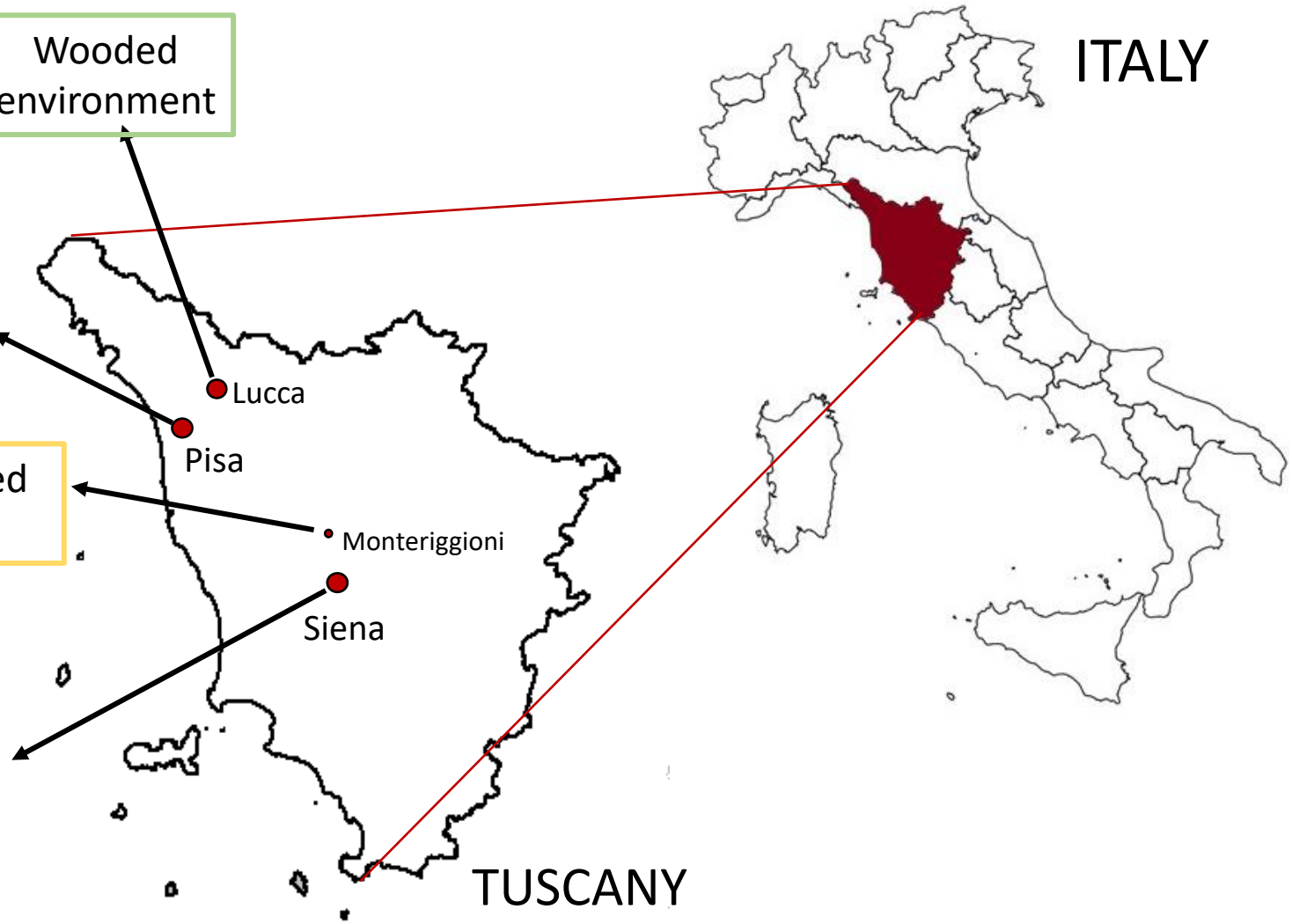
50 adults per area

Wooded environment

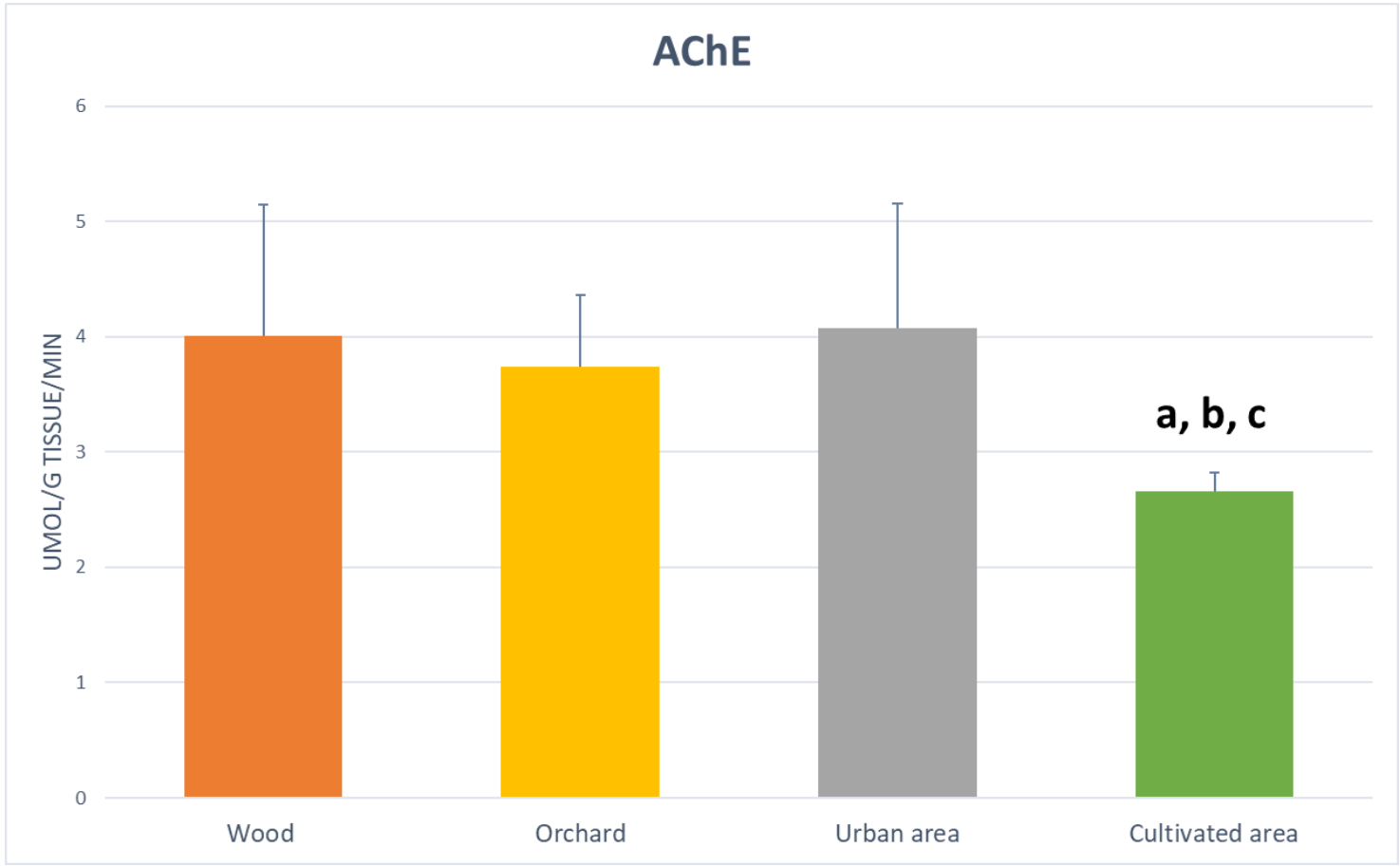
Orchard

Cultivated area

Urban site

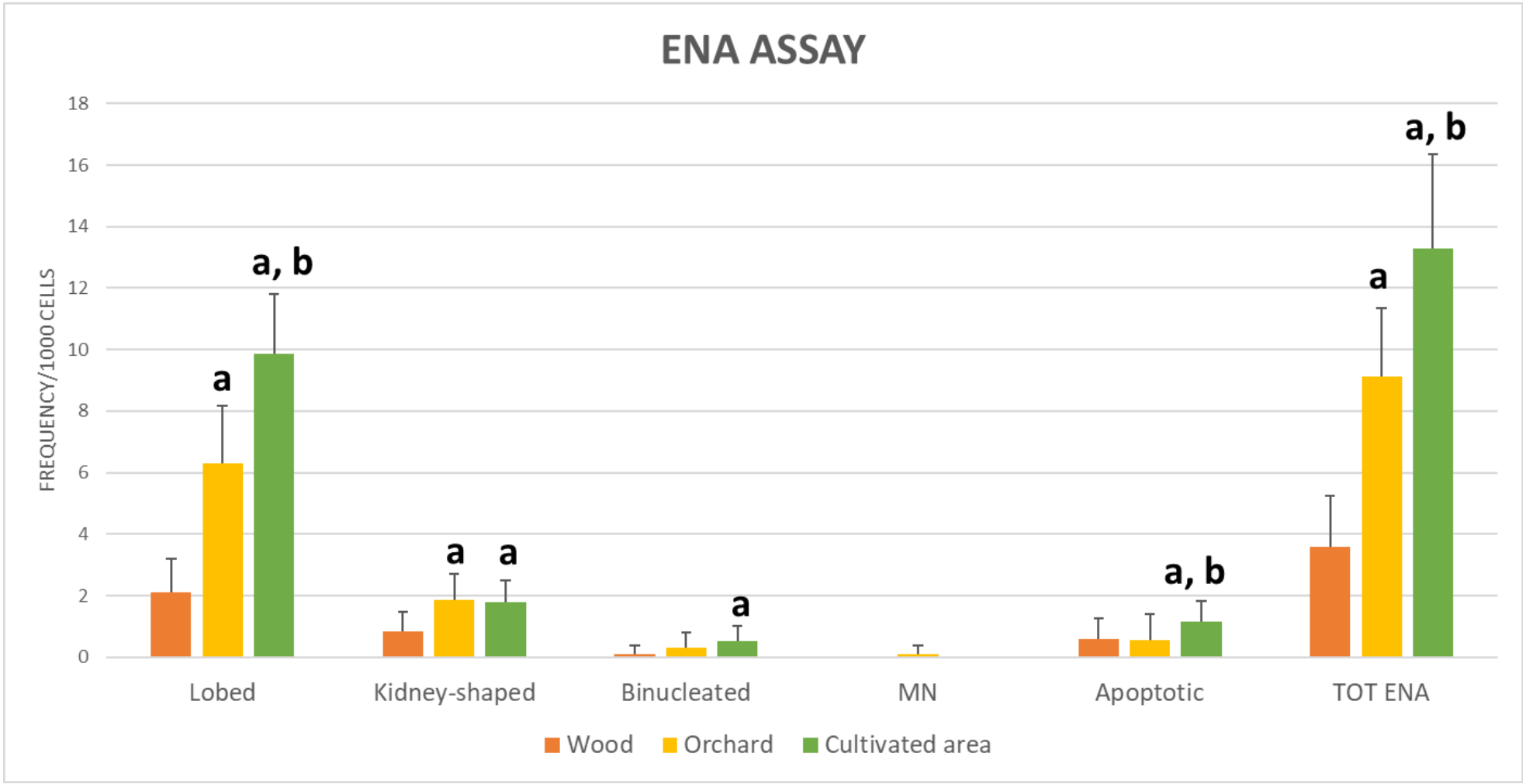


Biomarker of neurotoxicity



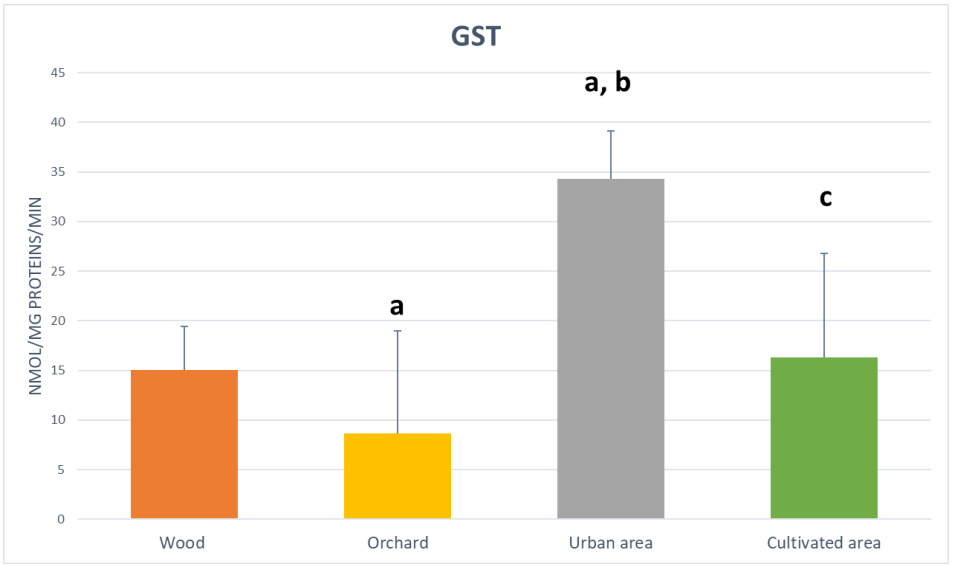
a indicates statistical difference in comparison with wood area ($p<0.05$)
b indicates statistical difference in comparison with orchard area ($p<0.05$)
c indicates statistical difference in comparison with urban area ($p<0.05$)

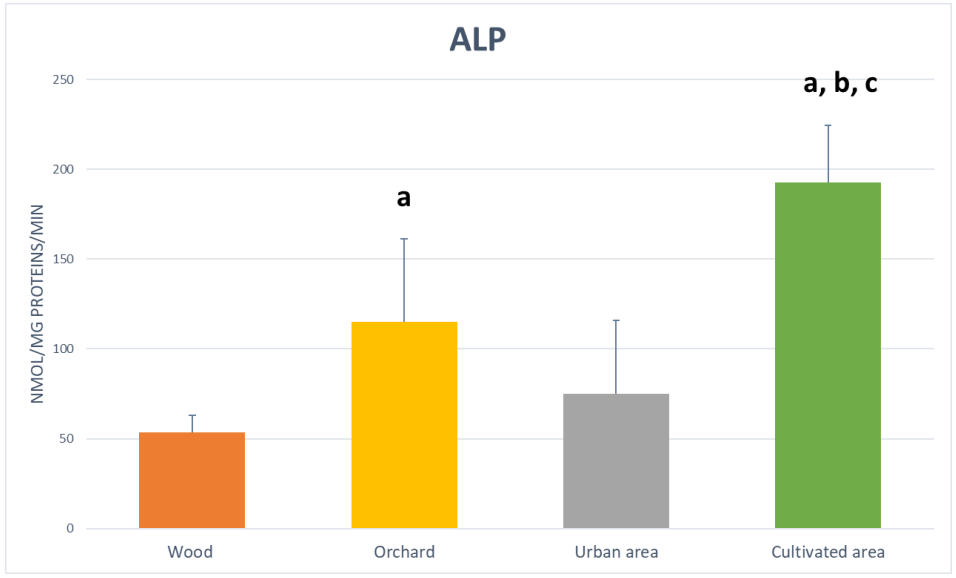
Biomarker of genotoxicity

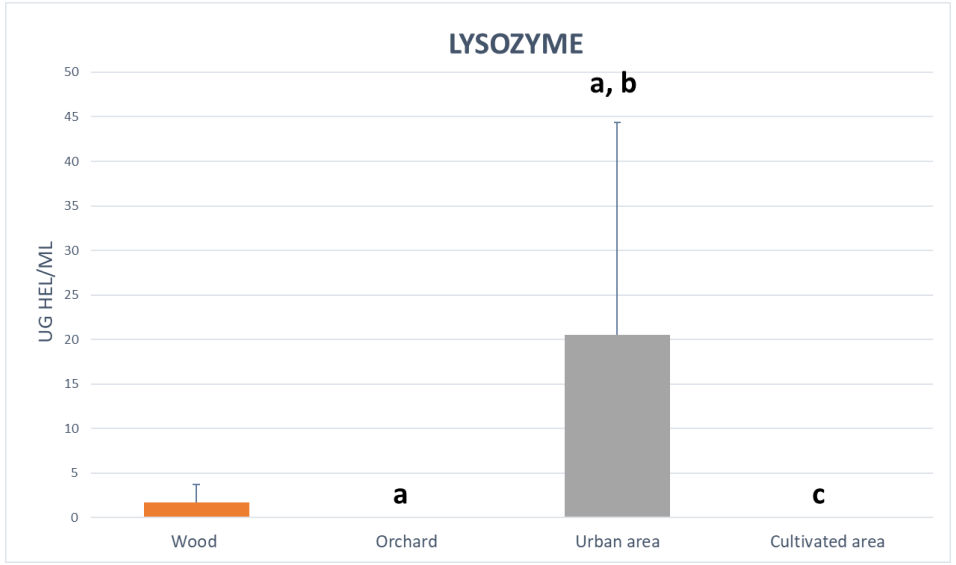


a indicates statistical difference in comparison with wood area ($p<0.05$)

b indicates statistical difference in comparison with orchard area ($p<0.05$)







a indicates statistical difference in comparison with wood area ($p<0.05$)
b indicates statistical difference in comparison with orchard area ($p<0.05$)
c indicates statistical difference in comparison with urban area ($p<0.05$)

Alterations with respect to the control area

CULTIVATED AREA

- Genotoxicity (Increase of erytrocytes nuclear abnormalities)
- Neurotoxicity (Inhibition of AChE activity)
- Metabolic alteration (induction of ALP enzyme)

ORCHARD

- Genotoxicity (Increase of erytrocytes nuclear abnormalities)
- Metabolic alteration (induction of ALP enzyme)

URBAN AREA

- Alteration of phase II enzymes (Induction of GST activity)
- Alteration of immune system (lysozyme)

Conclusions….

Need to extend the application of this approach and further develop it

Establish possible relation to contaminant levels and higher biological responses (behavioural, ecological…)

Useful approach for investigating sublethal effects of commercial pesticides (before commercialization ?)

Useful approach for investigating sublethal effects in field populations


Thanks for your attention!