

DANIELA LUPI¹, SILVIA CASINI², Francesca Mapelli¹, Elena Crotti¹, Sara Basiglio¹, Erica Holzer¹, Rida Ali¹, Francesco Patriarca¹, Ilaria Caliani², Tommaso Campani², Agata Di Noi³, Laura Giovanetti², Lisa Cabiddu⁴, Lucia Lenzi⁴, Mariana Paola Mazzei⁴, Francesco Lami⁴, Fabio Sgolastra⁴

¹Department of Food, Environmental and Nutritional Sciences, University of Milan, Milan, Italy

²Department of Physical, Earth and Environmental Sciences, University of Siena, Siena, Italy

³Santa Chiara Lab, University of Siena, Siena, Italy

⁴Department of Agricultural and Food Science, University of Bologna, Bologna, Italy

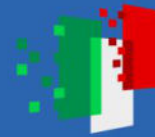
Chemical pollutants pose a significant threat to bee health, affecting their physiology, behavior, and survival. While the effects of specific insecticides on bees are well known, it is necessary to consider that bees are routinely exposed to complex mixtures of chemicals, including fungicides, herbicides, heavy metals, and veterinary drugs. The combined effects of these pollutants remain poorly understood, particularly their impacts at sublethal concentrations. Research on chemical exposure has largely been focused on honeybees, yet other pollinators, including solitary bees, also face similar risks. By integrating multiple stressors and diverse pollinator taxa, the study aims to expand our understanding of pollutant-pollinator interactions beyond the traditional honeybee model. A multidisciplinary approach has been adopted to provide valuable insights into bee survival considering and integrating information from different factors that are affected by pollutants. In particular, this research aims to evaluate effects of chronic oral exposure at sublethal concentration to different chemicals (boscalid, copper(II)chloride dihydrate; ivermectine and glyphosate) on survival of adults of the social honey bee (*Apis mellifera*) and the solitary red mason bee (*Osmia bicornis*), by integrating gut microbiome characterisation and different biomarker responses. As a result different chemicals impacted survival at different level. The gut microbial community of these pollinators was investigated by molecular analyses in terms of abundance and taxonomic composition, using quantitative PCR and high-throughput Illumina sequencing, respectively. Overall, the results indicate that all the administered chemicals, though to a different extent, affected the gut microbiome of bees in terms of community composition and diversity. As biomarkers, acetylcholinesterase, carboxylesterase, glutathione S-transferase, alkaline phosphatase, phenoloxidase and prophenoloxidase activities were evaluated, together with nuclear abnormalities assay and differential hemocytes count. In the solitary bee, detoxification processes were induced after both copper and boscalid+copper(II)chloride dihydrate treatments compared to control and boscalid ones. After ivermectine and ivermectine+glyphosate treatments, *Osmia bicornis* specimens showed metabolic alteration. Higher values of DNA damage were observed after all treatments, both alone and in combination. These findings will help refine environmental risk assessments and support conservation efforts to protect pollinators and their crucial ecosystem services.



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Daniela Lupi¹

Silvia Casini², Francesca Mapelli¹, Elena Crotti¹, Sara Basiglio¹, Erica Holzer¹, Rida Ali¹, Francesco Patriarca¹, Ilaria Caliani², Tommaso Campani², Agata Di Noi³, Laura Giovanetti², Lisa Cabiddu⁴, Lucia Lenzi⁴, Mariana Mazzei⁴, Francesco Lami⁴, Fabio Sgolastra⁴

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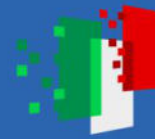
Chemical Pollution and Pollinator Health: Assessing Multi-Stressor Effects on Honeybees and Solitary Bees



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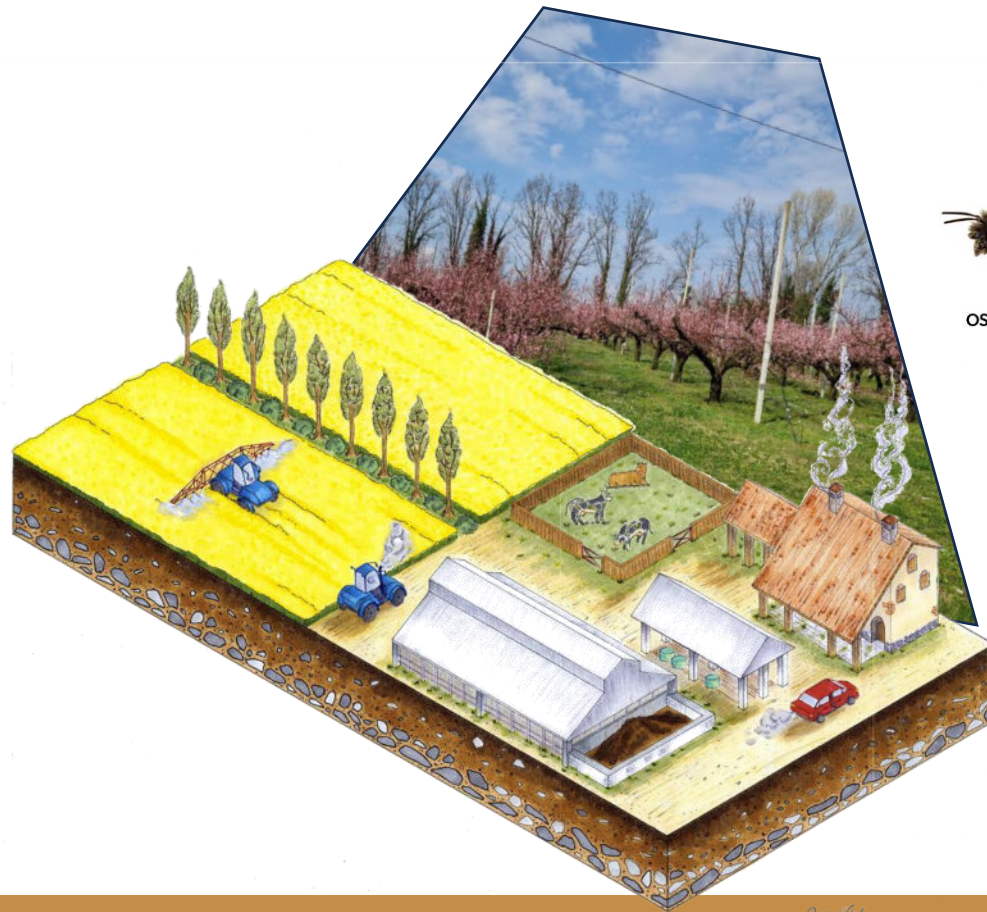
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OSMIA BICORNIS



APIS MELLIFERA



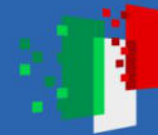
Dina Jovan



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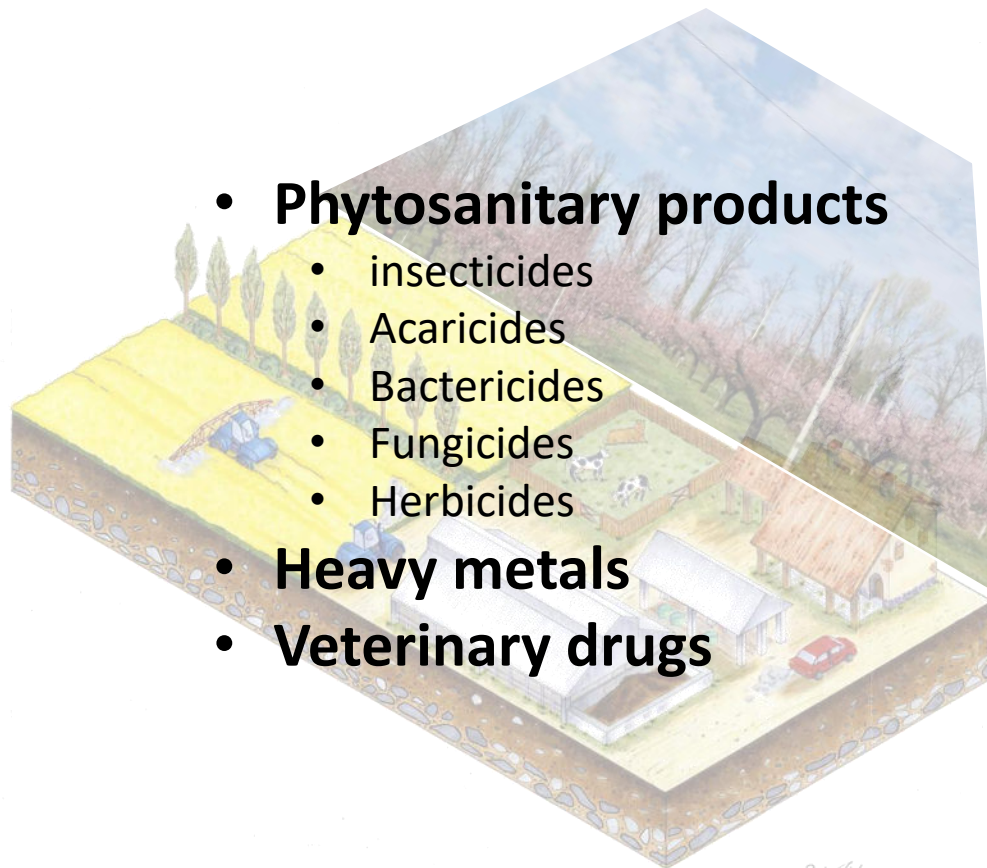
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- **Phytosanitary products**
 - insecticides
 - Acaricides
 - Bactericides
 - Fungicides
 - Herbicides
- **Heavy metals**
- **Veterinary drugs**



OSMIA BICORNIS



OSMIA BICORNIS



APIS MELLIFERA



APIS MELLIFERA

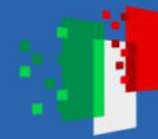
- **Phytosanitary products**
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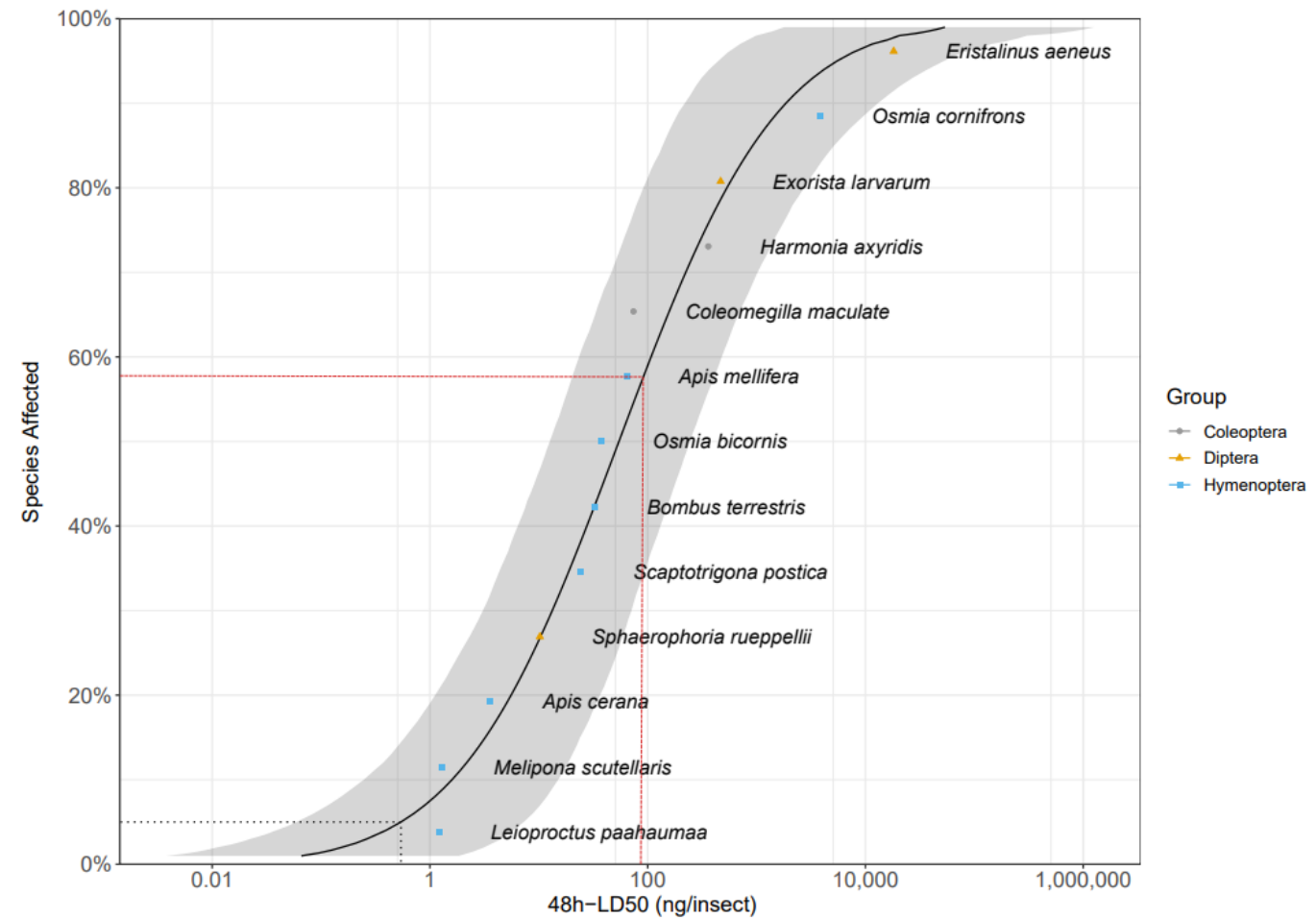


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- **250.000 pollinator species**
- Most papers → *Apis mellifera* (Arena et al., 2014)
- Same pollutant → Different **LD50**
- Most researches → **insecticides**
- OECD standards on *A. mellifera*

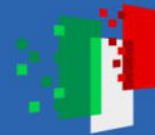




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Subchronic lethal doses

IMPAIRMENT



Lifespan Length

Nervous system and behaviour

Postembryonal development

Genotoxicity

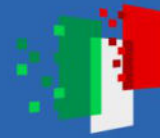
Microbiome impairment



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Aim of the study



Mason bee (*Osmia bicornis*),

solitary

Social



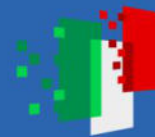
Honeybee (*Apis mellifera ligustica*)



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Aim of the study

Sublethal
Chronic doses
impairment

longevity

gut microbiome

biomarkers

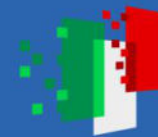




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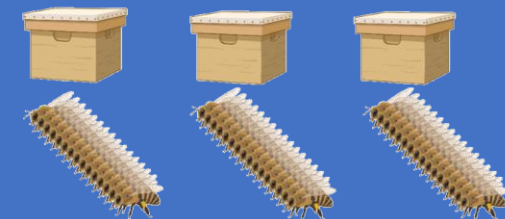
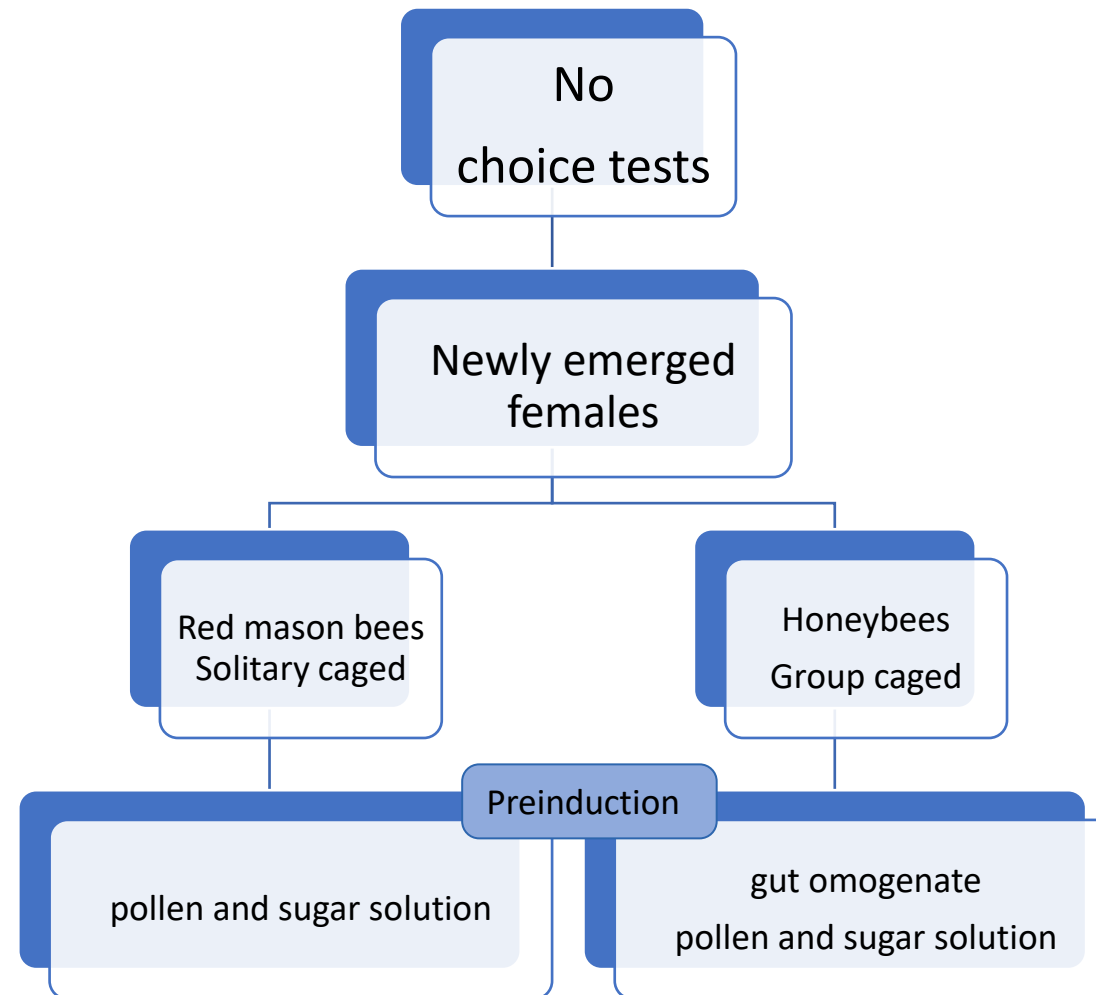


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Modified from

Azpiazu, C., Hinarejos, S., Sancho, G., Albacete, S., Sgolastra, F., Martins, C. A. H., ... & Bosch, J. (2023). Description and validation of an improved method to feed solitary bees (*Osmia* spp.) known amounts of pesticides. *Ecotoxicology and Environmental Safety*, 264, 115398.



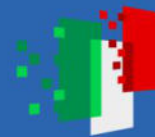
Modified from
OECD standards for chronic oral
toxicity test for *Apis mellifera*



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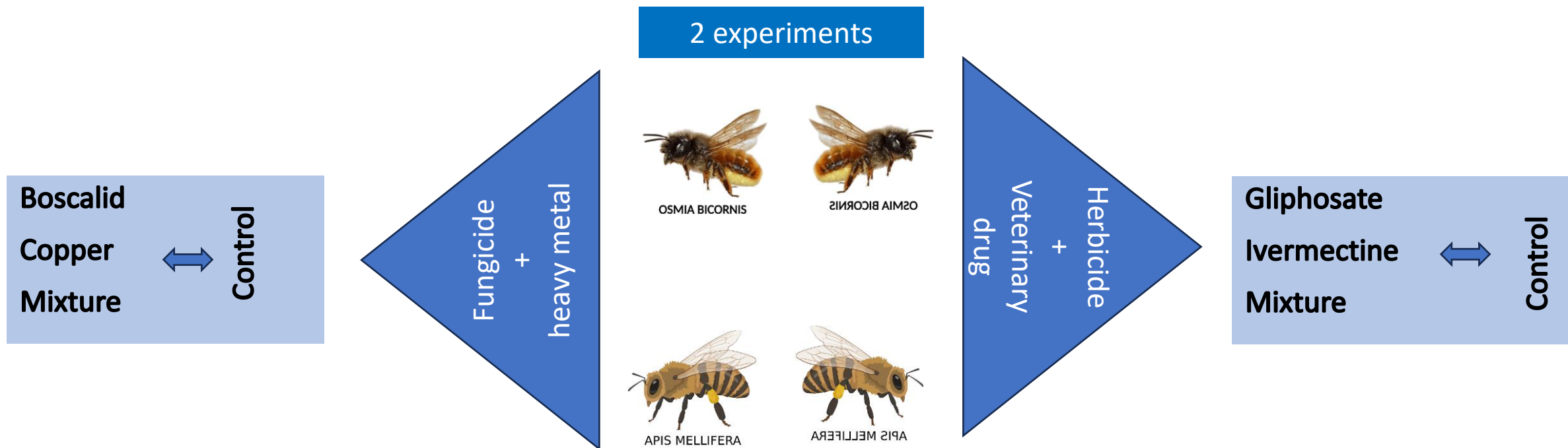


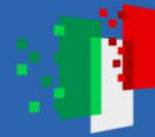
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Material and methods



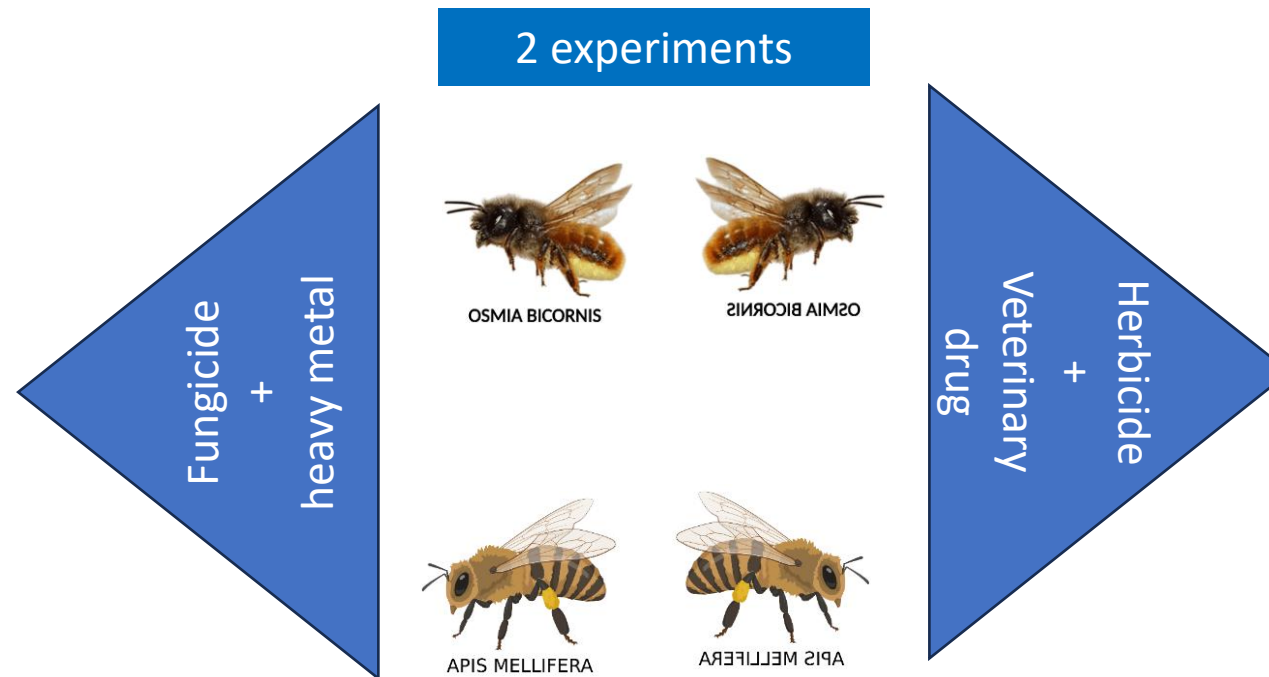


Boscalid

- **Fungicide**, mitochondrial respiration inhibitor
- detected it in nectar up to **1.43 mg/kg**
- **Disrupt of the gut microbiota at sublethal doses** (Dong et al., 2023)
- Concentration tested: **2 mg/Kg** corresponding to 1/10 of LC50 (Walner, 2009)

Copper (II) chloride dihydrate

- **Fungicide**, water soluble and used in previous studies (Lewis et al., 2016)
- **LC50 → 72 mg/L** (Di et al., 2016)
- **Sublethal effects at 1/10 LC50** (Nikolić et al., 2016)
- Concentration tested: **7.2 mg/Kg**



Glyphosate

- **Systemic herbicide** with a broad spectrum
- **Inhibit the production of aromatic aminoacid** in plants
- Concentration tested: **30 mg/Kg** (Thompson et al. 2014)

Ivermectine

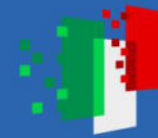
- Anthelmintic and acaricide **veterinary drug**
- acts on the nervous system
- Concentration tested: **0.16 µg/ml** corresponding to 1/10 of the LC50 in *A. mellifera* (Guseman et al., 2016)²



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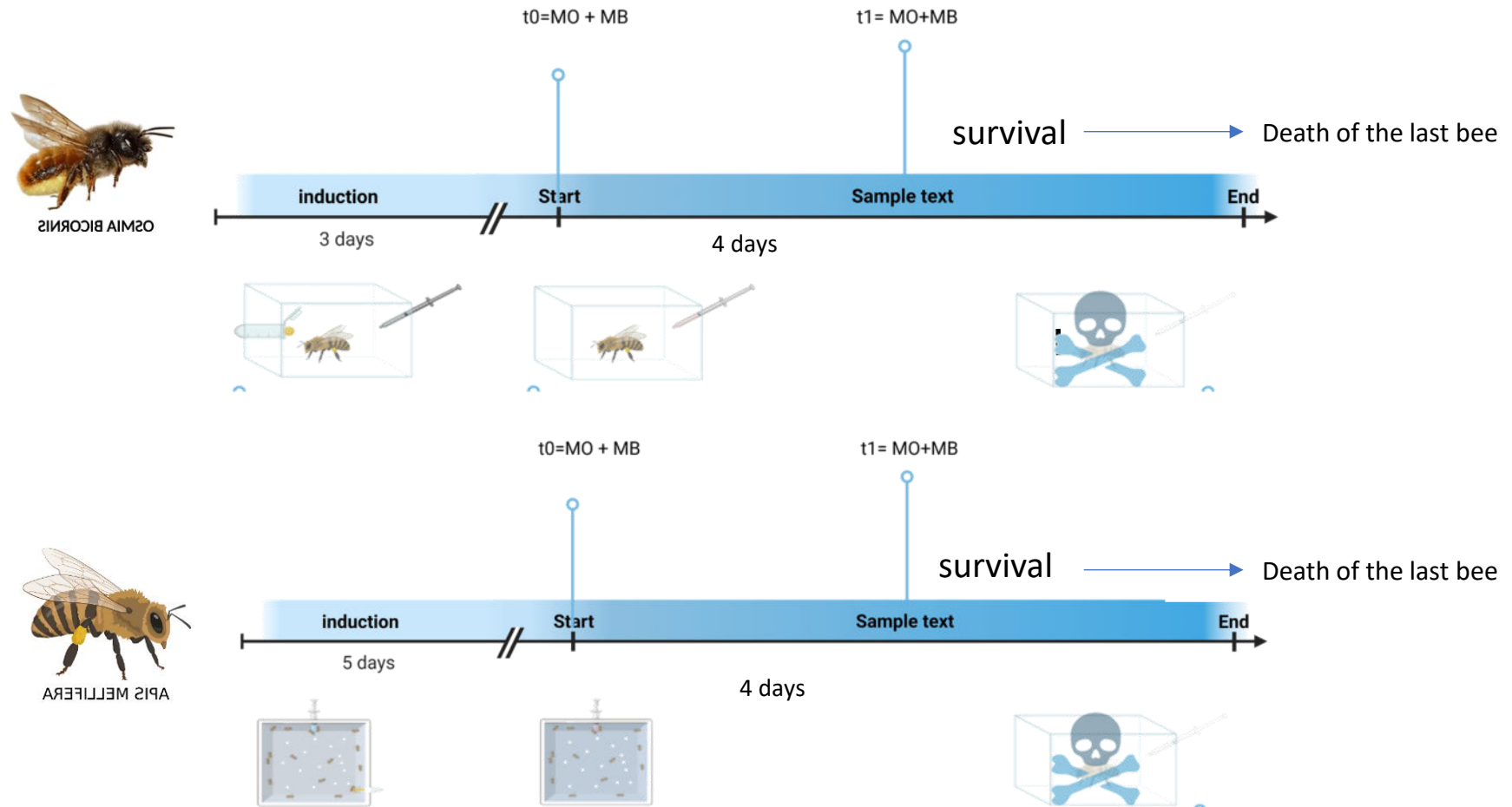


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TIMELINE

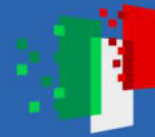




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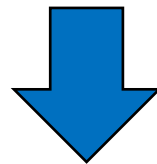
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MICROBIOME ANALYSIS

- 1) Superficial Sterilization
- 2) Gut Dissection



1) Dna Extraction

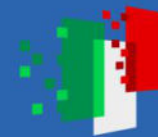
- ☐ Quantification of total bacterial abundance: qPCR-16S Rrna
- ☐ Study of bacterial community composition using NGS sequencing of the 16S rRNA gene (Illumina)



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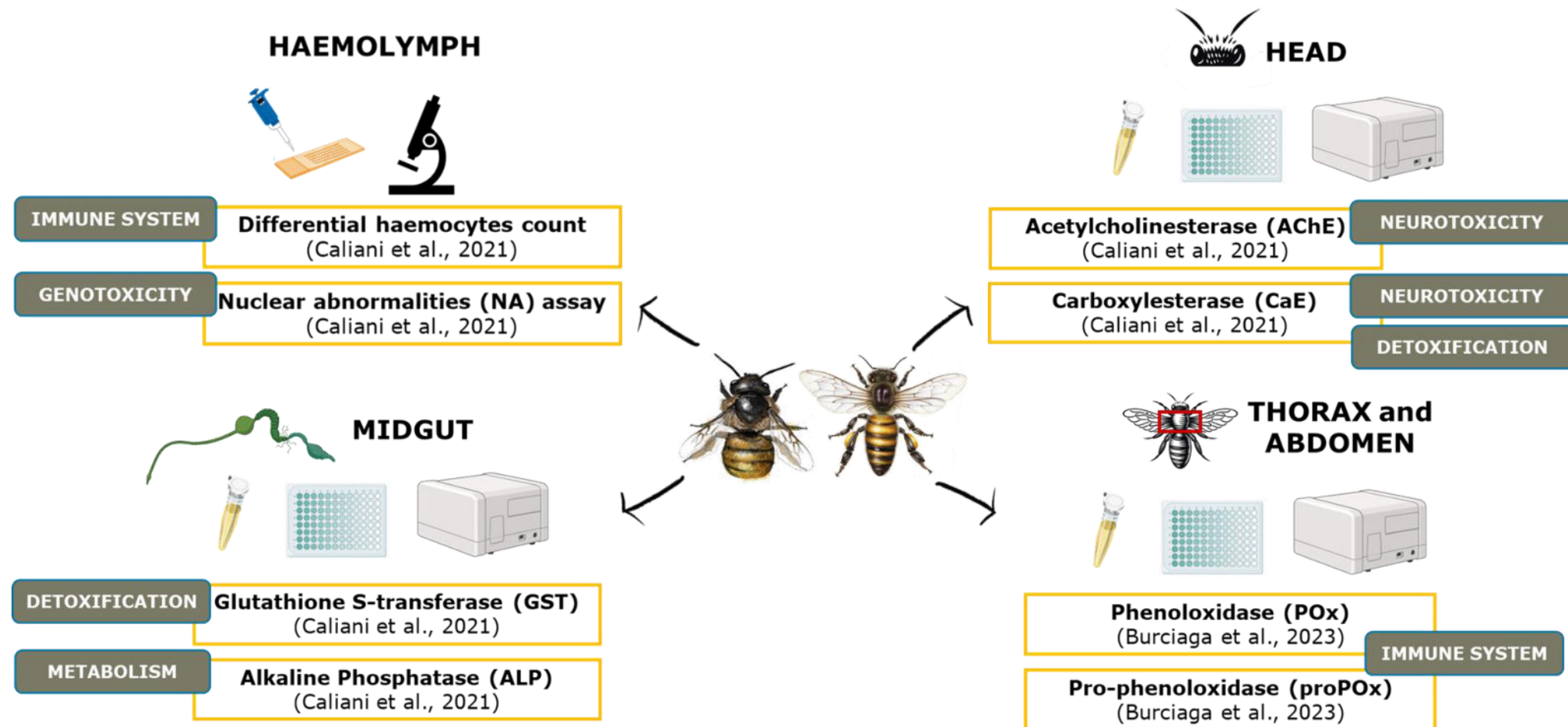
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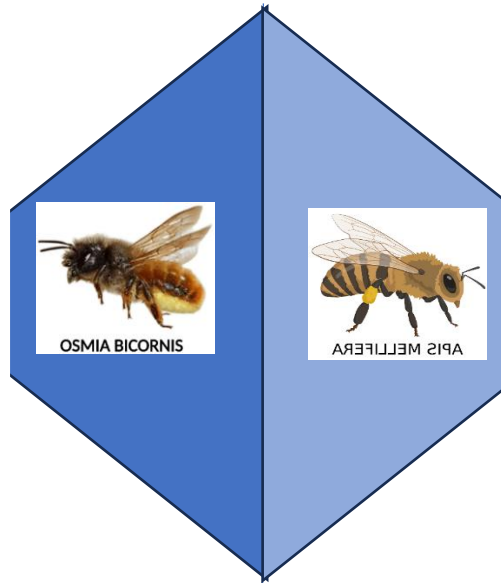
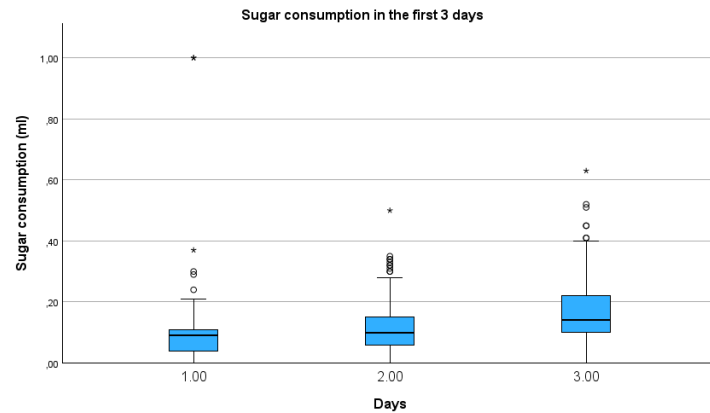
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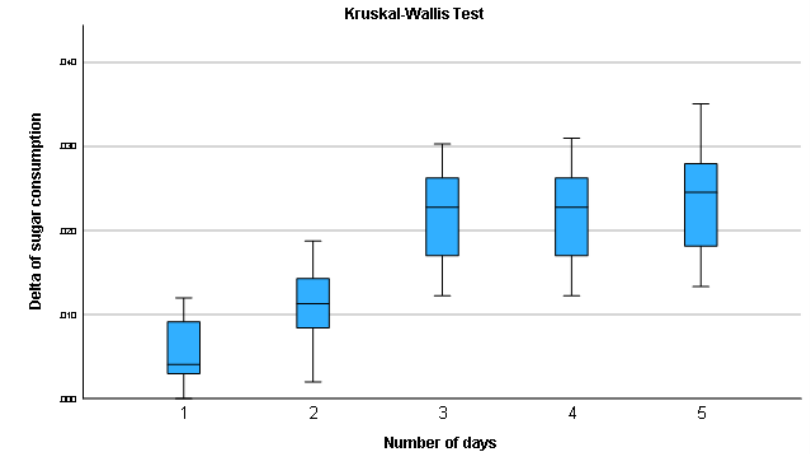
BIOMARKER ANALYSIS

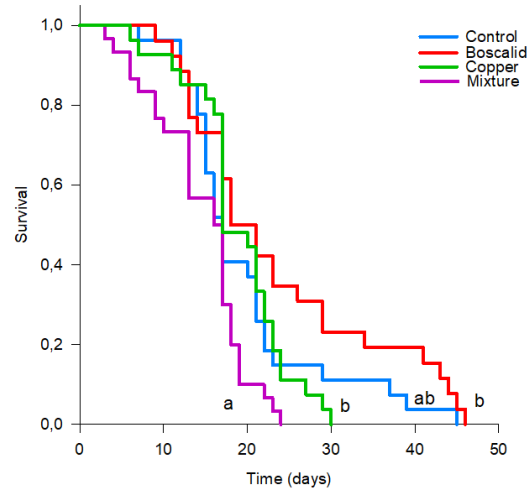


Preinduction consumption



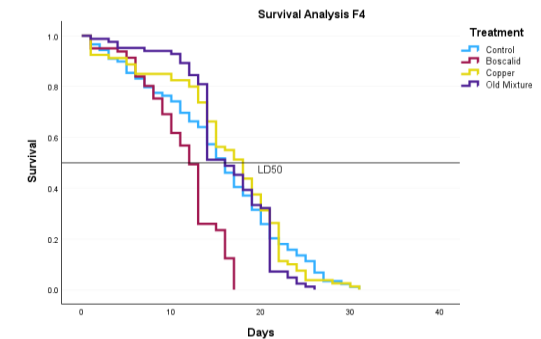
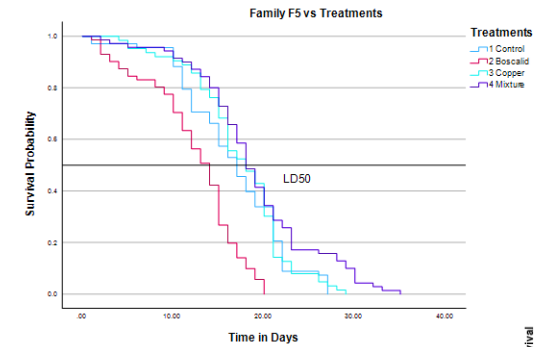
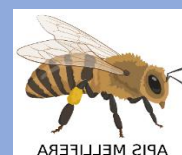
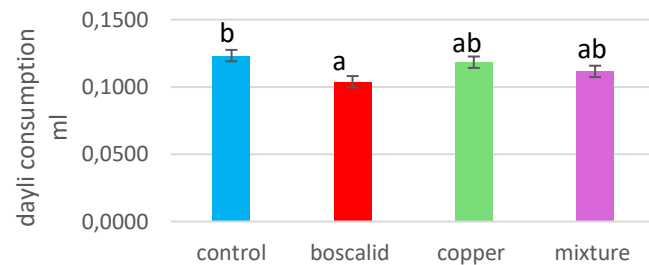
Increase in sugar consumption
from day 1 to day 3 in all
treatments





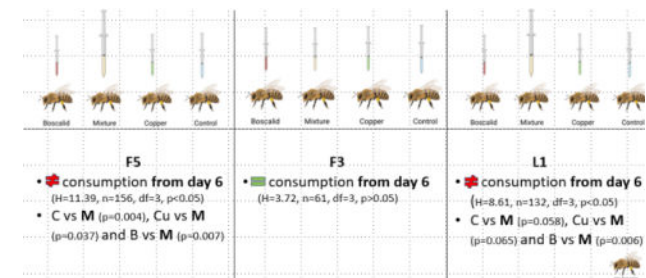
(Kaplan-Meier; Log-rank test; $p < 0.001$)

- Survival affected by the combination of 2 chemicals (Mixture)



- Survival affected by boscalid

(Kaplan-Meier; Log-rank test; $p < 0.001$)



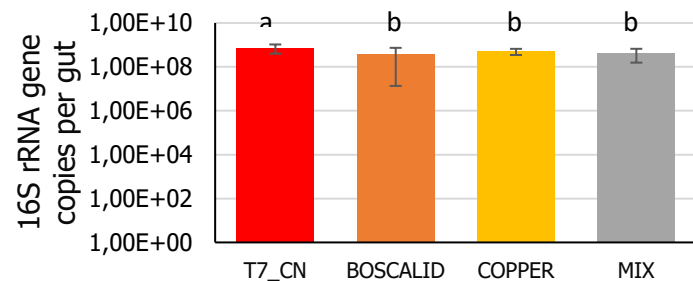
- Consumption differed with the origin of the bees

Fungicides + heavy metal

Results
Survival

Results
Microbiome

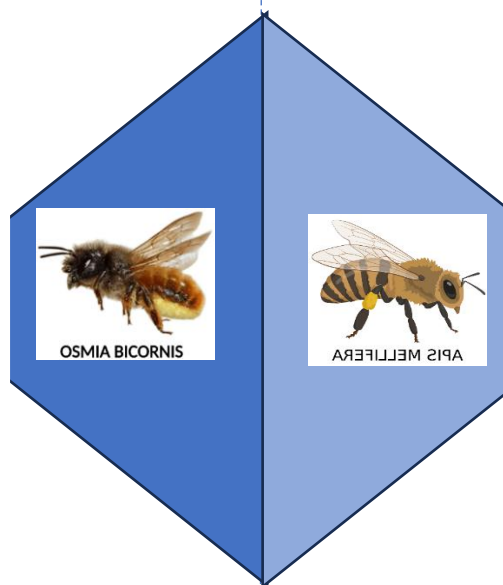
Bacterial abundance



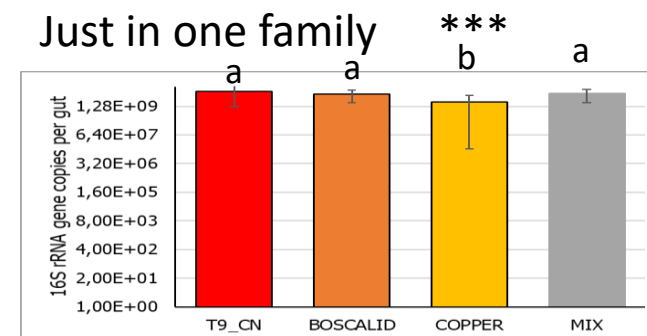
$p < 0.05$ (Kruskall-Wallis, Dunn tests)

Fungal abundance

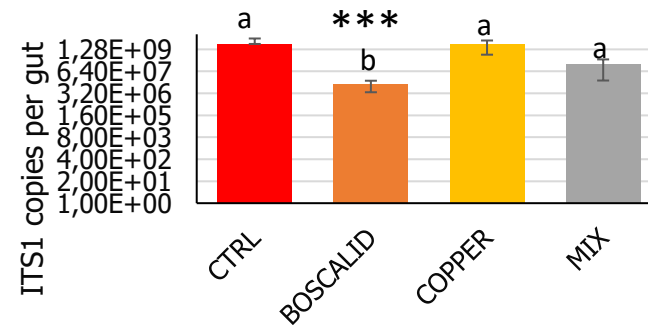
NO significant differences in gut fungal abundance



Just in one family



qPCR on 16S rRNA gene



Graphs from qPCR on ITS1 fragment

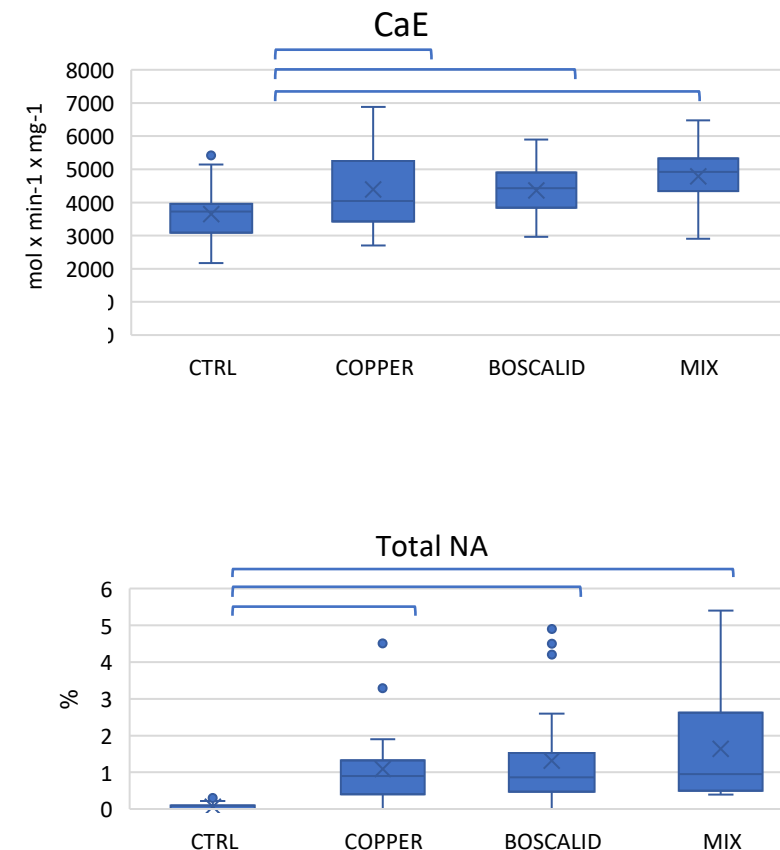
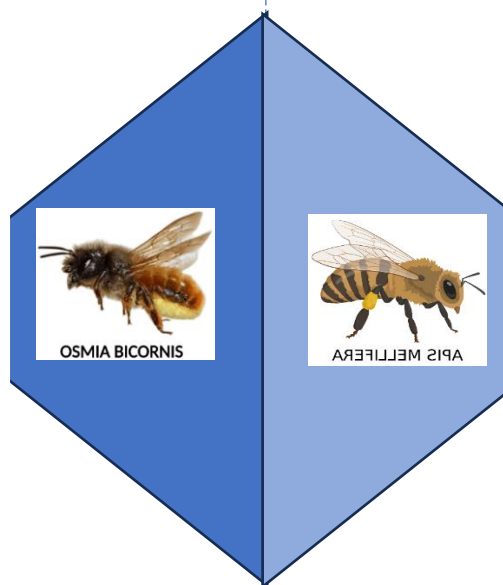
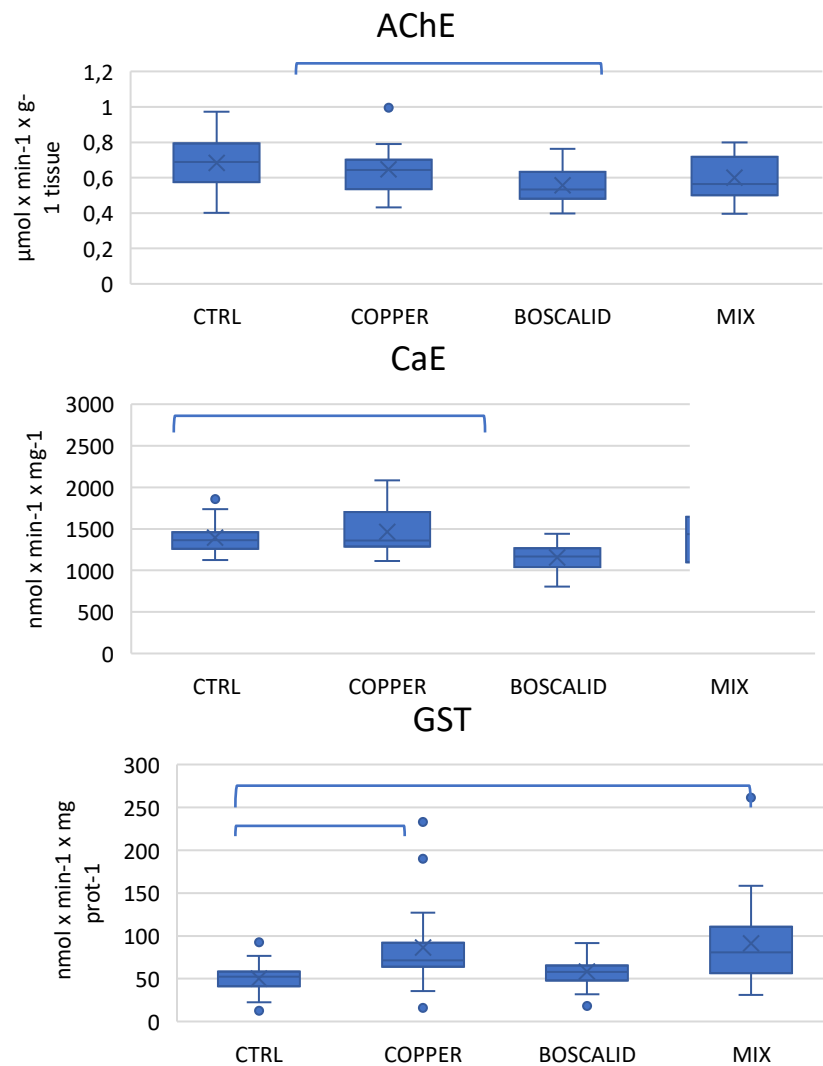
Fungicides + heavy metal

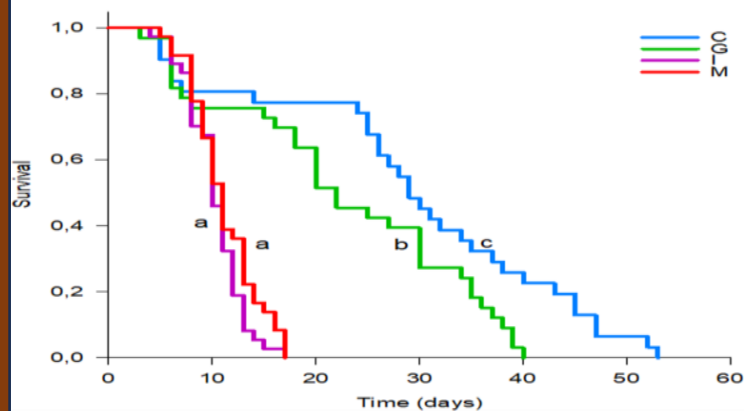
Results Survival

Results Microbiome

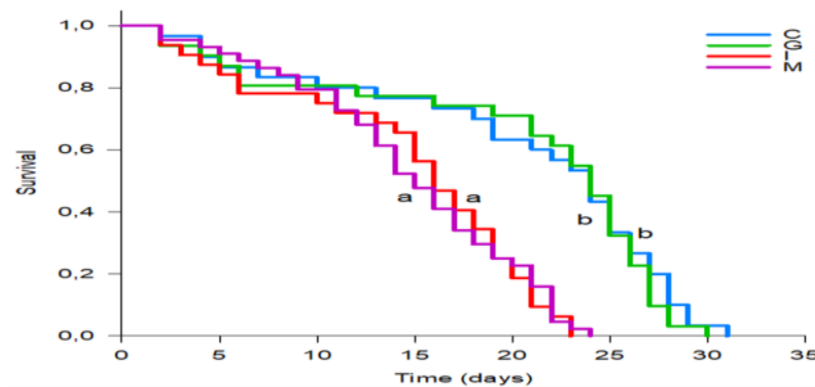
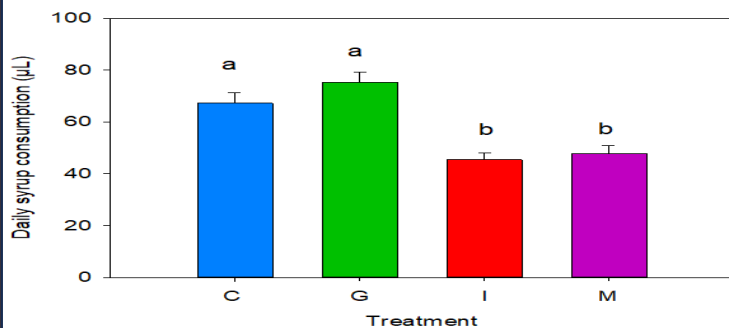
Results Biomarkers

FIRST EXPERIMENT





- Survival affected by ivermectine and Mixture
(Kaplan-Meier; Log-rank test; $p < 0.001$)



- Survival affected by ivermectine and Mixture
(Kaplan-Meier; Log-rank test; $p < 0.001$)

NO significant differences in consumption

Glifosate+ ivermectine

Results
Survival

Results
Microbiome

Bacterial abundance

NO significant differences in gut bacterial abundance

NO significant differences in gut bacterial abundance

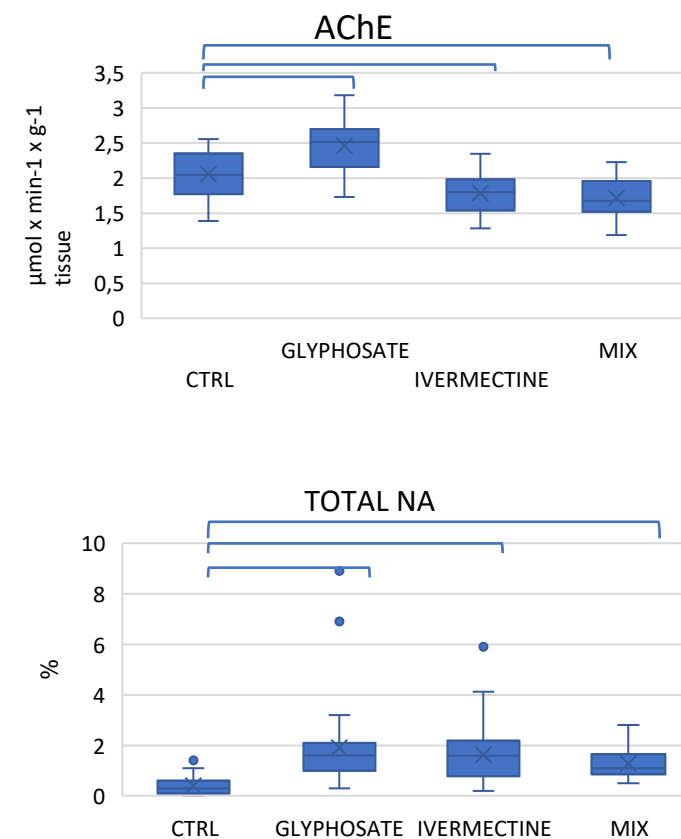
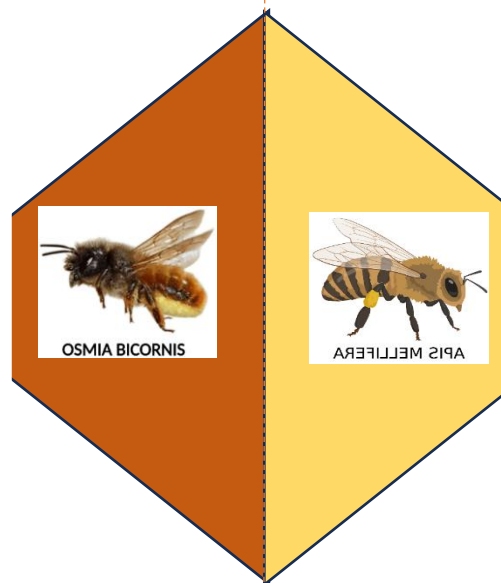
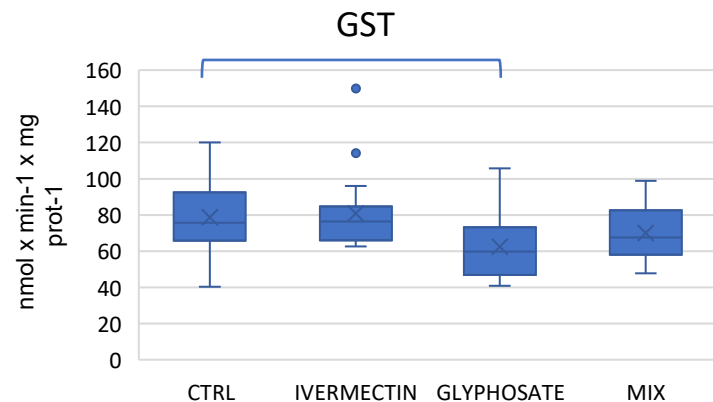
Fungal abundance

NO significant differences in gut fungal abundance

NO significant differences in gut fungal abundance



SECOND EXPERIMENT

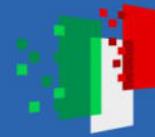




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Take home message

- **Different treatments** had **distinct effects** on bees survival.
- *Apis mellifera* and *Osmia* showed sometimes different responses, **reflecting species-specific sensitivity**.
- **Survival** effects became **evident mainly in the long term**, underscoring the importance of assessing chronic sublethal doses.
- Alterations were also observed in the **microbiome**.
- **Biomarker** analyses confirmed these patterns, with specific active ingredients affecting both biomarkers and survival.

						
	boscalid	coppert	mix	Glyphosate	Ivermectine	mix
Survival			✓		✓	✓
Microbiome	✓	✓	✓			
Biomarkers	✓	✓		✓	✓	✓

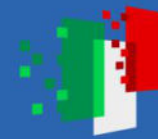
Need to consider effects also on other wild bees and different endpoints



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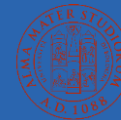
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THANK YOU FOR YOUR ATTENTION!!



Contact: daniela.lupi@unimi.it



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