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Istituto Universitario di Studi Superiori, Pavia

BIOCHEMICAL AND CELLULAR ALTERATIONS OF A MIXTURE OF GLYPHOSATE AND AMINOMETHYLPHOSPHONIC ACID IN MUSSELS *Mytilus galloprovincialis*

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INTRODUCTION

Glyphosate (GLY) is among the most widely used non-selective systemic herbicides at global scale, detected in all environmental matrices along with its main breakdown product aminomethylphosphonic acid, AMPA[1]. After the use, these organophosphorus compounds can reach water bodies from in-land sources and interact with non-target aquatic organisms, causing concerns for the possible ecotoxicological effects on wild biota and risks for environmental and human health[2].

Despite being recognized as toxic for aquatic species[3], glyphosate effects and mechanisms of action in non-target species are yet unclear and scarcely documented, and its use has been recently renewed in EU until 2033[4].

AIM

- Explore the biological alterations caused by GLY and AMPA, using the Mediterranean mussel *Mytilus galloprovincialis* as non-target aquatic model organism.
- Investigate mechanisms of interaction between tested compounds in binary mixture.
- Assess the persistence of biological alterations after a recover period.
- Provide synthetic hazard indices through a weighted elaboration of a wide panel of biological endpoints, according to their toxicological relevance following a Weight of Evidence approach.

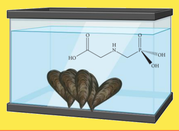
MATERIAL AND METHODS

EXPERIMENTAL DESIGN

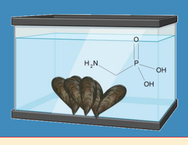
Adult mussels (5.3 ± 0.4 cm shell length) were exposed for **28 days** in duplicate tanks, each containing 30 organisms, to environmentally realistic concentrations of GLY, AMPA and their mixture; after exposure, mussels were left to recover for **14 days.**

CONTROL (CTL)
(clean artificial seawater)

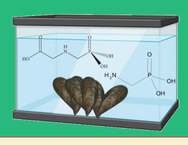
GLYPHOSATE (GLY)
(0.5µg/L)



AMPA (AMP)
(0.5µg/L)



MIX (GLY + AMP)
(0.5µg/L + 0.5µg/L)

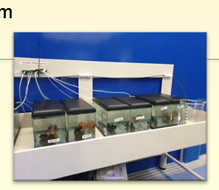


Water change every other day and chemicals re-dosed

Daily fed with phytoplankton

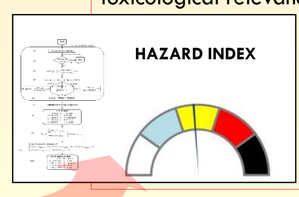
INVESTIGATED CELLULAR SYSTEMS

- Immunocytes sub-populations and functionality
- Cholinergic response
- Xenobiotic metabolism
- Detoxification mechanisms
- Antioxidant system and oxidative damages
- Lipid metabolism
- DNA damage



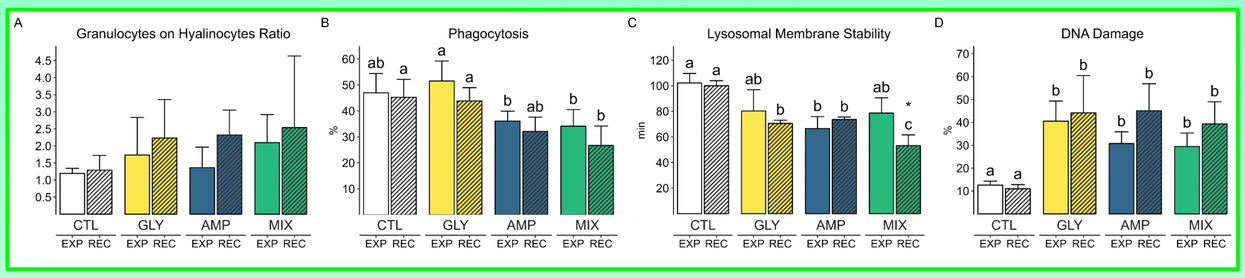
WEIGHT of EVIDENCE ELABORATION

Results of biological responses were elaborated through a quantitative Weight of Evidence approach to summarize the hazard associated to each experimental conditions, based on toxicological relevance of measured biological endpoints, statistical significance and magnitude of observed variations compared to specific thresholds. The elaborated Hazard Quotients (HQ) are then assigned to one of five classes of hazard, from Absent to Severe.

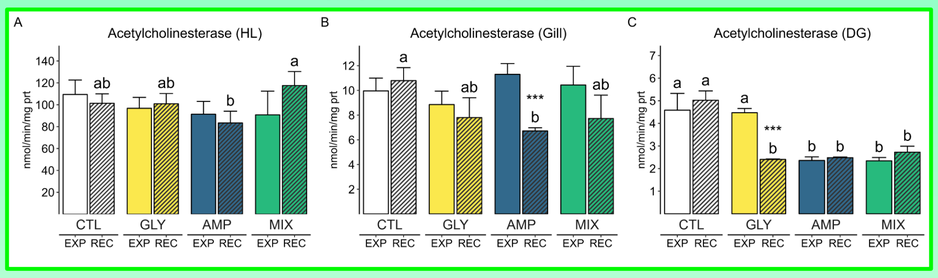


RESULTS

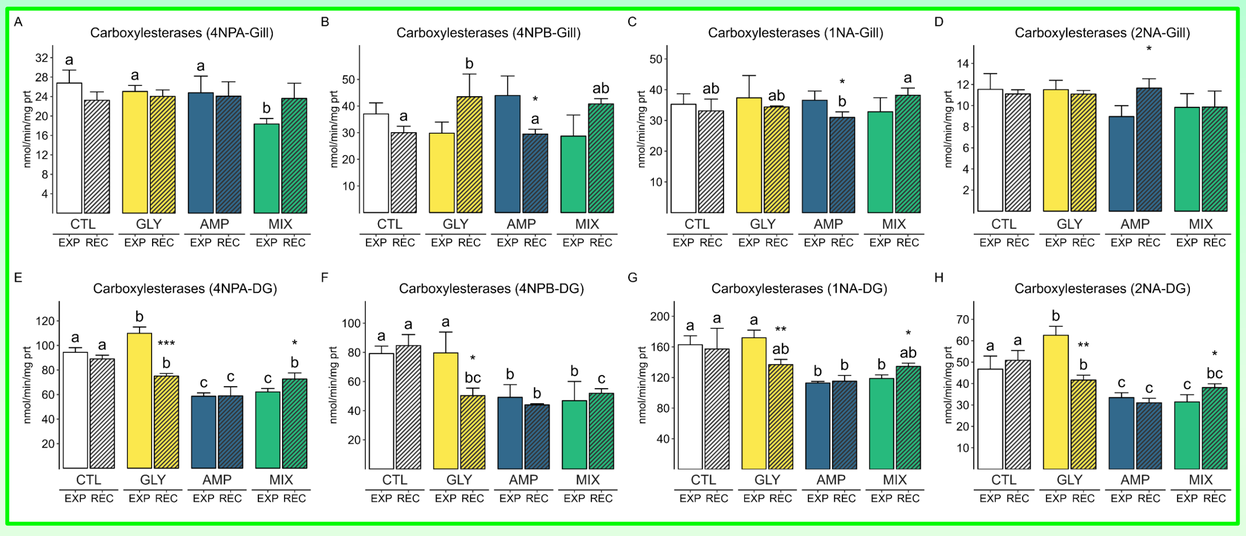
IMMUNOCYTES RESPONSES



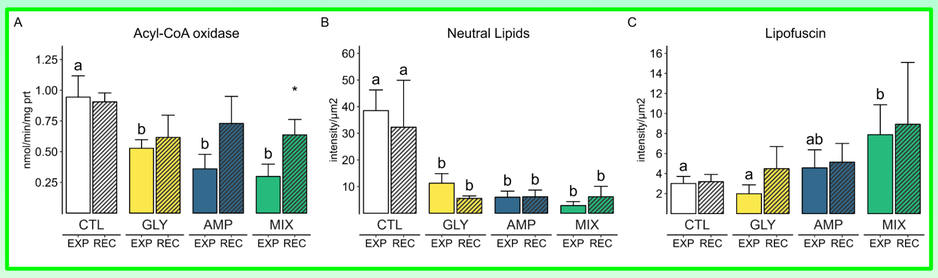
CHOLINERGIC FUNCTION



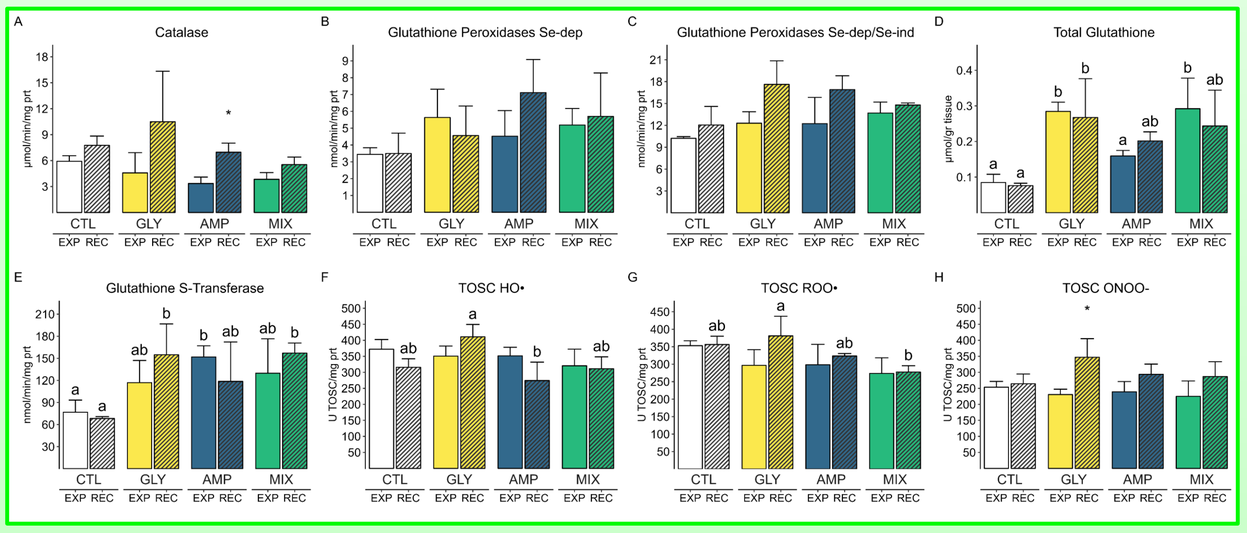
XENOBIOTIC METABOLISM



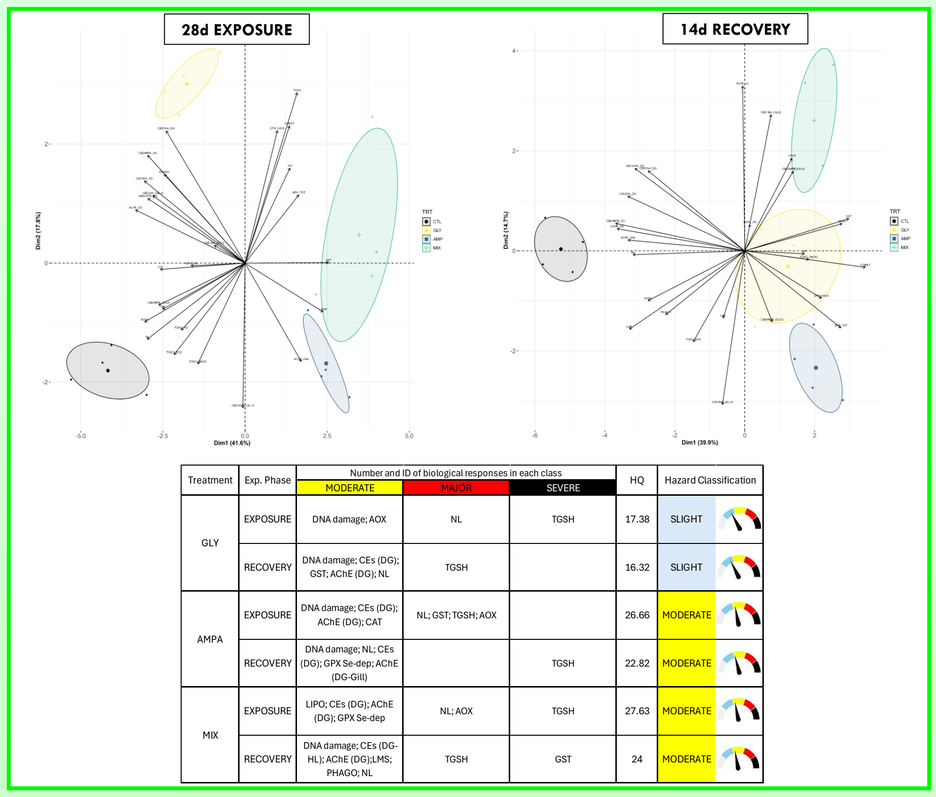
LIPID METABOLISM AND PEROXIDATION



ANTIOXIDANT DEFENSES AND OXIDATIVE DAMAGES



PRINCIPAL COMPONENTS ANALYSIS and WEIGHTED OF EVIDENCE ELABORATION



Treatment	Exp. Phase	Number and ID of biological responses in each class: MODERATE	MAJOR	SEVERE	HQ	Hazard Classification
GLY	EXPOSURE	DNA damage; AOX	NL	TGSH	17.38	SLIGHT
	RECOVERY	DNA damage; CEs (DG); GST; AChE (DG); NL	TGSH		16.32	SLIGHT
AMPA	EXPOSURE	DNA damage; CEs (DG); AChE (DG); CAT	NL; GST; TGSH; AOX		26.66	MODERATE
	RECOVERY	DNA damage; NL; CEs (DG); GPX Se-dep; AChE (DG-Gill)		TGSH	22.82	MODERATE
MIX	EXPOSURE	LIPO; CEs (DG); AChE (DG); GPX Se-dep	NL; AOX	TGSH	27.63	MODERATE
	RECOVERY	DNA damage; CEs (DG-HL); AChE (DG);LMS; PHAGO; NL	TGSH	GST	24	MODERATE

Letters are used to highlight significant differences between treatments in the same experimental phase, while asterisks (*) are used to highlight significant differences between exposure and recovery phases of each treatment.

HIGHLIGHTS

- AMPA significantly reduced phagocytosis (in AMP- and MIX-exposed mussels).
- Lysosomal membrane destabilization was observed in all experimental treatments, in particular after recovery.
- Onset of DNA damage was observed at the end of the experiment and remained after recovery.
- Limited responsiveness of antioxidant system was observed in organisms exposed to GLY and AMPA, either alone or in combination.
- Activation of the phase II glutathione S-transferase activity was observed in all experimental treatments in both phases, coupled to total glutathione increase, suggesting onset of cellular detoxification mechanisms through conjugation of GSH to tested compounds and its consumption.
- AMPA significantly inhibited cholinergic function in digestive gland (in AMP- and MIX-exposed mussels) at the end of the exposure, persisting despite the 14-days recovery.
- A generalized inhibition of carboxylesterases activity (phase I detoxification mechanism), was observed in digestive gland of mussels exposed to all experimental treatments in both phases.
- Limited disturbance of AChE in haemolymph and gills, mainly at the end of the recovery phase.
- A consistent decrease of Acyl-CoA oxidase activity in all treatments after the exposure, coupled to mobilization of stored neutral lipids, highlighted lipid metabolism disruption by all tested compounds, persisting despite the recovery.
- Accumulation of lipofuscin in MIX-exposed mussels suggest cumulative pro-oxidant mechanisms targeting cellular components.
- Overall, PCA showed a clear separation between CTL groups and tested compounds, confirming their biological reactivity and suggesting a limited recover capability of non-target species.
- The weighted elaboration assigned a higher hazard classification to AMP- and MIX-treatments (“MODERATE”) compared to GLY (“SLIGHT”) in both experimental phases.
- The environmental health implications deriving from glyphosate may not be strictly related to the parent compound but rather from its breakdown product.

REFERENCES

1. Matozzo et al., 2019. Ecotoxicological hazard of a mixture of glyphosate and aminomethylphosphonic acid to the mussel *Mytilus galloprovincialis* (Lamarck 1819)
2. Pagano et al., 2024. Assessment of oxidative stress biomarkers in the threatened annual killifish *Austrolebias charrua* exposed to Roundup
3. ECHA, 2022. Glyphosate. European Chemicals Agency, Helsinki, Finland
4. Commission Implementing Regulation (EU) 2023/2660 of 28 November 2023

ACKNOWLEDGEMENTS

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