

Combined Effects of Microplastics and Trace Metals on Stress-Response Gene Expression in the Mediterranean Mussel *Mytilus galloprovincialis*

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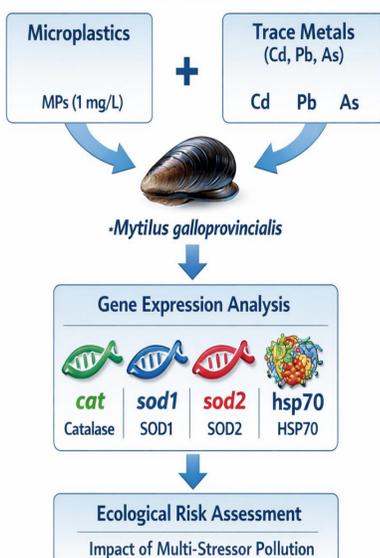
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INTRODUCTION & AIM

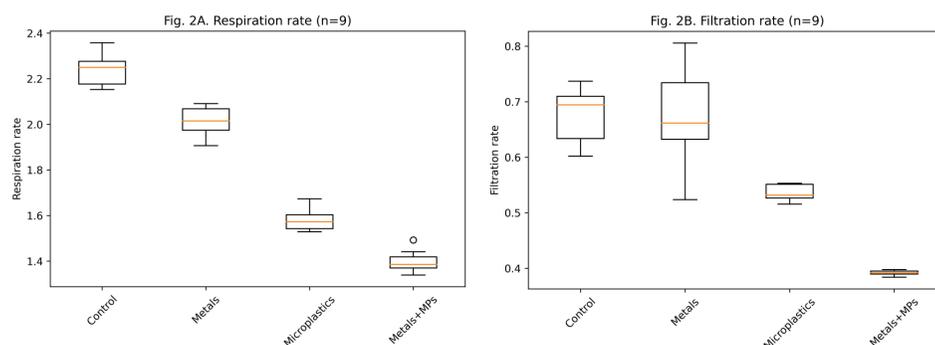
The Mediterranean Sea is widely recognized as one of the most contaminated semi-enclosed marine basins and constitutes a major hotspot for microplastic and trace metal contamination. Microplastics (MPs) are widely distributed in Mediterranean surface waters and sediments, where their concentrations rank among the highest reported globally (Terzi et al., 2024). Beyond their direct physical impacts, MPs can adsorb trace metals onto their surfaces, thereby potentially modifying metal bioavailability and toxicity (Rasool et al., 2025). The Mediterranean mussel, *Mytilus galloprovincialis*, is an ecologically and economically important species in Mediterranean coastal ecosystems and is extensively used as a bioindicator in marine monitoring programs. Due to their filter-feeding activity and pollutant bioaccumulation capacity, bivalves are frequently exposed to complex mixtures of contaminants rather than individual stressors. Although the effects of MPs and trace metals have been investigated separately, their combined influence on molecular stress responses in *M. galloprovincialis* remains insufficiently understood, particularly under environmentally relevant Mediterranean conditions.

Study Objectives



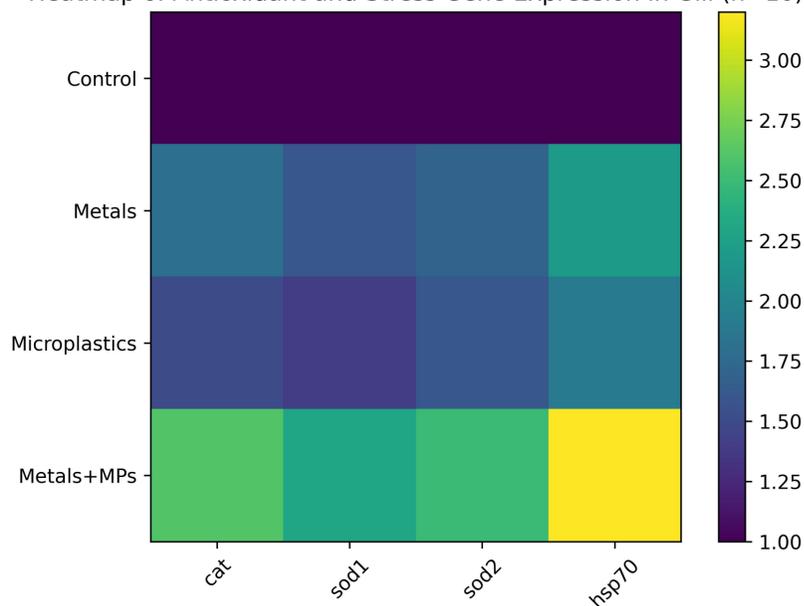
RESULTS & DISCUSSION

1. Changes in filtration and respiration rates



2. Pathway genes expression in mussel gill

Heatmap of Antioxidant and Stress Gene Expression in Gill (n=10)



METHOD

Test Organisms & Acclimation

Mytilus galloprovincialis
27g WW; 4cm SL

- Uncontaminated
- 35 PSU, 18°C
- 12h Light:12h Dark
- Fed daily w/ microalgae
- Seawater renewed 48h

7 DAYS ACCLIMATION

Experimental Design

7 DAYS ACCLIMATION

14 DAYS SEMI-STATIC EXPOSURE

Control (C): 1 mg/L	Microplastics (MP): (10 µg/L 10 each)
Metals (M) Cd, Pb, As (10 µg/L 10 µg/L each)	MP + M + Cd, Pb, As + (Pd, µ2)3 µg/L each)

- 4 Groups
- 4 Groups
- ≥3 Replicate Tanks/treatment
- Media renewed 24-48h

Tissue Sampling

Gill
Digestive Gland

After 14 days exposure

- Tissues excised (Gill, Digestive Gland)
- Frozen in liquid nitrogen
- Stored at -80°C

Preparation of Exposure Solutions

Microplastics
Virgin (polystyrene/ polyshyline)
Sonicated 10-15 min.

Stock diluted to 10 mg/L in seawater

Trace Metals
Track diluted Purity (A220/280; & Integrity 18-2.1) (Gel Electrolites)
Cock diluted Elctst/L esals
Combined treatment: MP first, then metals

Molecular Analysis Workflow

Tissue (50-100 mg)

RNA Extraction (TRNOL₂) (TRIzol)

cuDA Synthesis (1 µg total RNA)

Quantitative Real-Time PCR (qPCR)

Quantitative Realtime PCR (qPC)

- Target Genes: cat, sod1, sod2, hsp70
- Rarget Genes: actin, aod2,
- Reference Gertin, 18S rNa, eflA
- SYBR Green chemistry
- Amplification: 40 aneening
- Melt curve analysis
- Technical triplicates

CONCLUSION

Microplastics and trace metals, both common in the Mediterranean Sea, can disrupt key stress and antioxidant genes in *Mytilus galloprovincialis*. While each pollutant alone triggered a response, their combination caused the strongest upregulation of gene expression and molecular stress signals. This suggests that microplastics may enhance metal toxicity, increasing risks for coastal marine life. Our findings highlight the need to consider pollutant mixtures in environmental monitoring.

Future translocation and epigenomic studies are needed to determine whether these stress responses persist across environments and potentially across generations.

FUTURE WORK / REFERENCES

1-Terzi, Y., Gündoğdu, S., Tekman, M. B., Gedik, K., Ustaoglu, D., Ismail, N. P., ... & Aydın, İ. (2024). How much do we know about the microplastic distribution in the Mediterranean Sea: a comprehensive review. *Marine Pollution Bulletin*, 208, 117049.

2-Rasool, A., Halfar, J., Brožová, K., Čabanová, K., Chromíková, J., Malíková, P., ... & Heviánková, S. (2025). Interactions of microplastics with heavy metals in the aquatic environment: Mechanisms and mitigation. *Journal of Hazardous Materials Advances*, 100984.