USING BIOMARKERS TO IDENTIFY OXIDATIVE STRESS RESPONSE IN ZEBRAFISH *(Danio rerio)* FED WITH MICROPLASTICS

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PLASTIC POLLUTION FROM MACRO TO NANO

Introduction

Fish living in their environment can be used as bioindicators of environmental pollution. This is crucial in understanding a potential contamination related to environmental conditions. In fact, they are directly exposed to chemicals and pollutants such as microplastics, which represent an increasing problem today. An oxidative stress levels variation is one of the possible consequences that those animals face and tool to understand the effects of pollutants on fish' physiology. Enzymes as catalase (CAT), glutamate S-transferase (GST), lipid peroxidation (LPO) and the functioning of the electronic transport system (ETS) could be important biomarkers fundamental to understand any possible imbalance which leads to the generation of ROS (reactive oxygen species). Thus, analyzing how the enzymatic defense mechanisms vary along the exposure period to contaminants leads us to a better understanding of a possible interaction between microplastic and fishes.

Methods

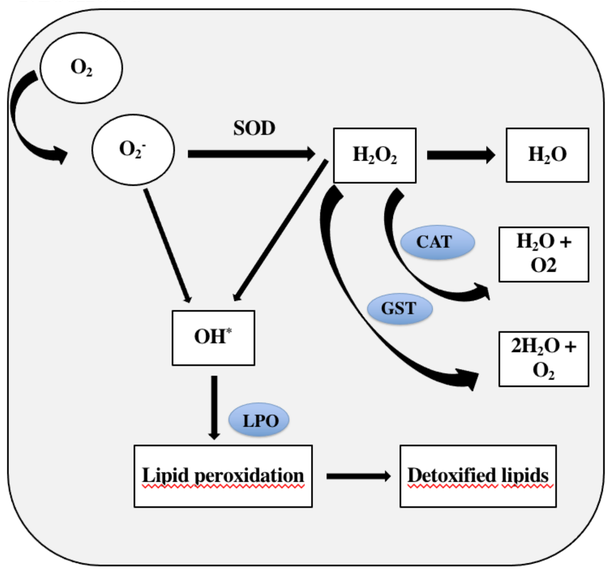


Fig. 1 Illustration representing the antioxidative ROS remotion promoted in cells by the studied enzymes (CAT, GST); and LPO indicating oxidative damage.

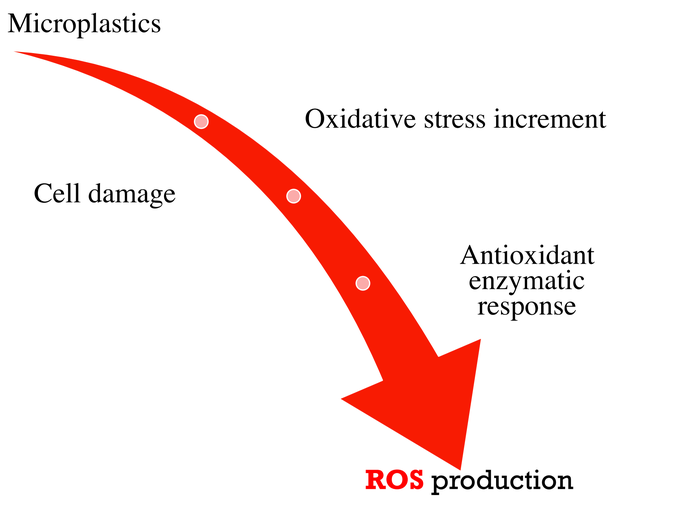


Fig. 2 Representation of the experimental process from the introduction of MPs in the diet to the consequences of oxidative stress.

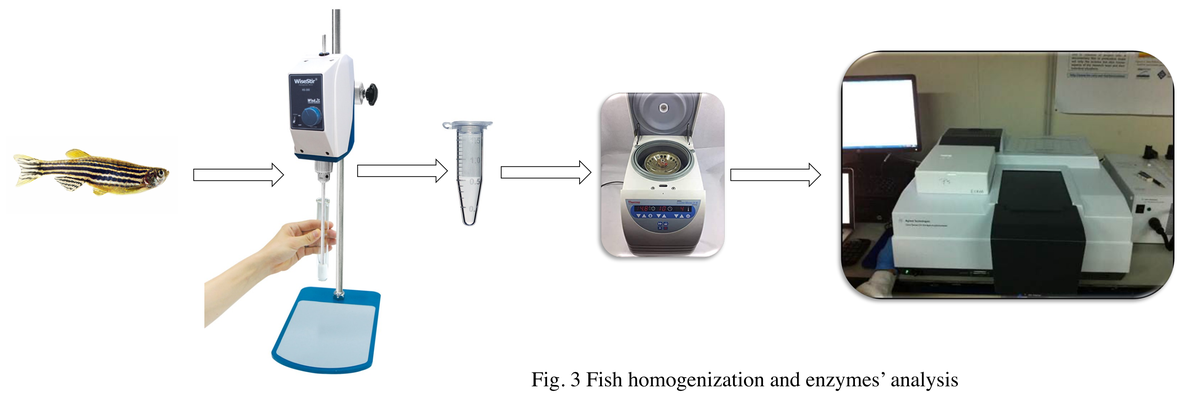
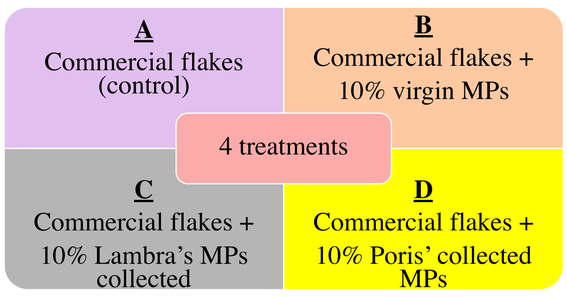


Fig. 3 Fish homogenization and enzymes' analysis



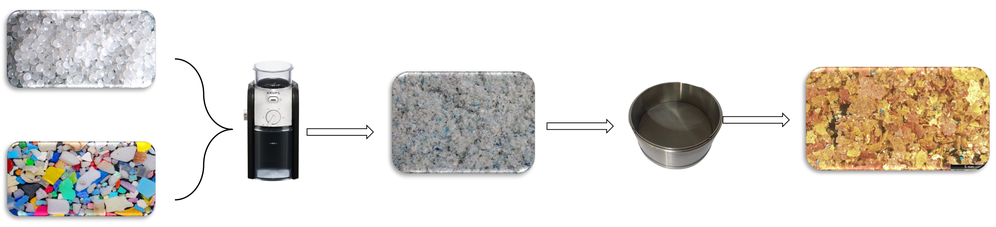
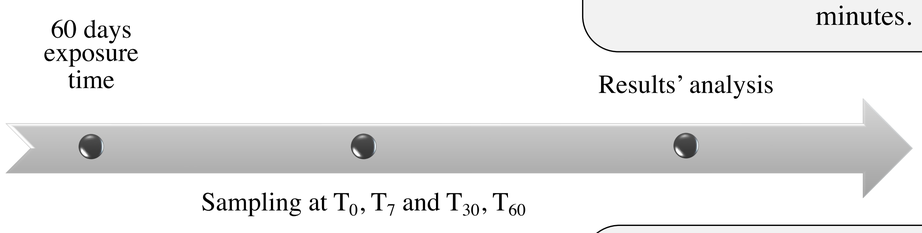


Fig. 4 Representation of the experimental process of plastic trituration



Catalase (CAT)

Aebi (1984); Demarchi et al. (2020)

- Sample + substrate solution + buffer;
- Reading absorbance at 240nm during 3 minutes.

Electron Transport System (ETS)

Owens and King (1975); Gómez et al. (1996)

- Sample + substrate solution + INT
- Reading absorbance kinetically at 490 during 8 min.

Statistical Analysis

Data were elaborated with SPSS (vers. 26). One-way ANOVA procedure was used for statistical analysis.

Glutathione s-transferase (GST)

Frasco & Guilhermino, (2002); Habig et al., (1974)

- Sample + substrate solution + buffer;
- Reading absorbance at 340 nm during 5 minutes.

Lipid Peroxidation (LPO)

Barboza et al. (2020)

- Sample + TCA (12%) – vortex;
- 240μl TRIS-DTPA + 300μl TBA (0.73%);
- 100°C – during 1 hour;
- Centrifugation at 11500rpm during 5' at 4°C;
- Reading absorbance at 535nm.

Results

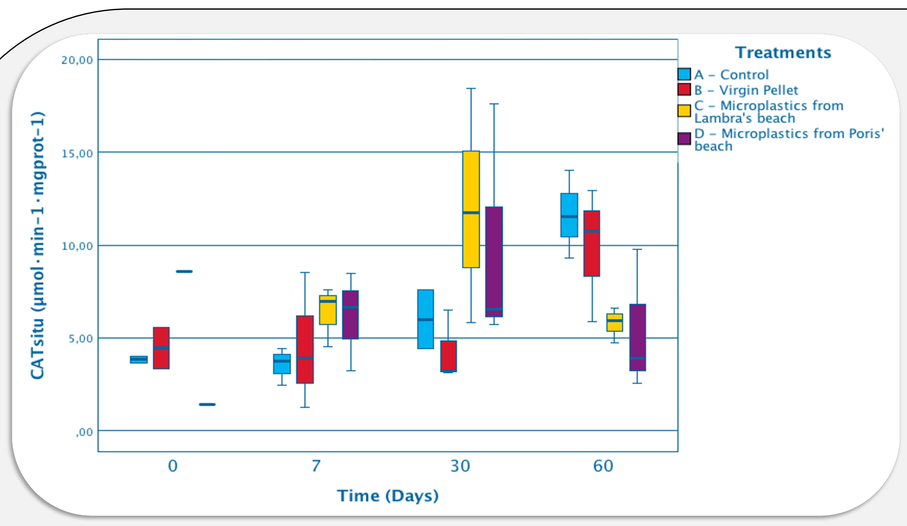


Fig. 5 Catalase activity (CAT)

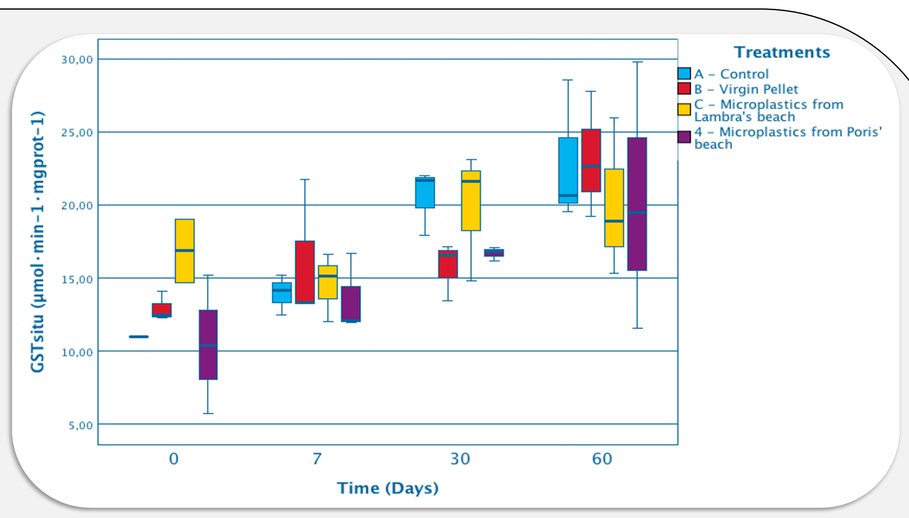


Fig. 6 Glutathione S-transferase activity (GST)

Enzymes variation along the experiment

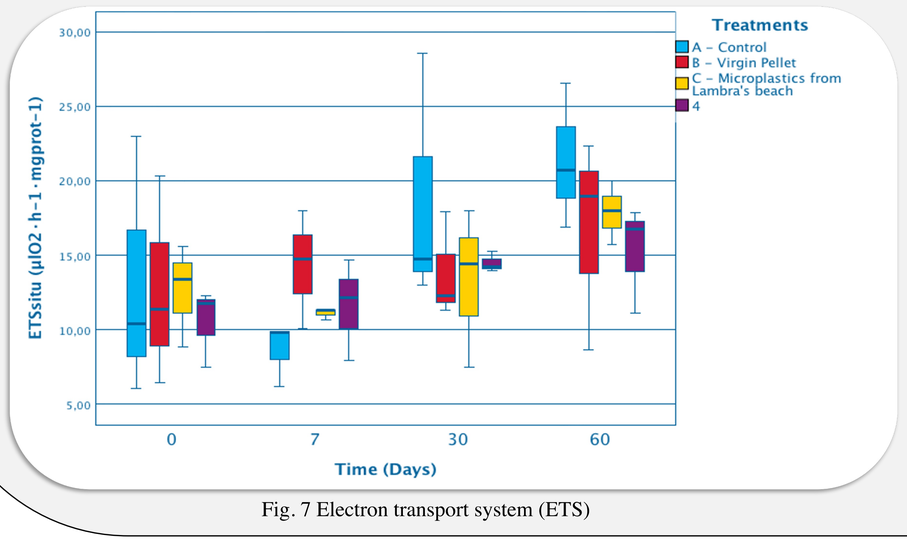


Fig. 7 Electron transport system (ETS)

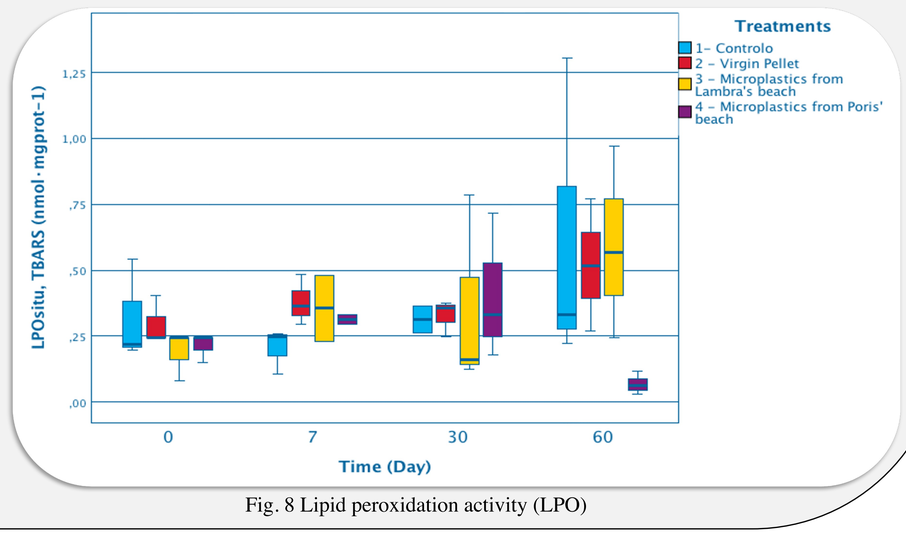


Fig. 8 Lipid peroxidation activity (LPO)

Conclusions

✓Concerning the **CAT** activity, we can assume that the **enzyme decrease** (for treatment C & D) might be related to an **enzyme disfunction** caused by an **oversaturation** of **ROS levels** (seen at T_{30})

✓A no significant difference between treatments ($p > 0.05$) in CAT, GST, LPO and ETS does not necessarily mean that microplastics are not affecting the fish organism

✓More detailed investigation is needed over different time periods and with different treatment variations (microplastic concentration and the amount of feedings per day)

✓More investigation is needed to acquire more knowledge about antioxidant enzyme variations related to microplastic exposure

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Acknowledgments

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