Alteration of cellular stress parameters on fish tissues, after "aged" and "virgin" microplastics ingestion

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Introduction

Plastic waste has become a serious problem in the natural environment and microplastic (MP) pollution has received increased attention over the last few years. Once MPs are ingested by aquatic organisms they tend to accumulate and have the potential to cause adverse effects, leading to inhibition of growth and development, reduction of feeding activity, energy disturbance, endocrine disruption, oxidative stress, cell apoptosis, which in turn leads to tissue damage, inflammation by interfering with immune system components, genotoxicity, neurotransmission malfunction and even mortality [1,2,3,4,5].

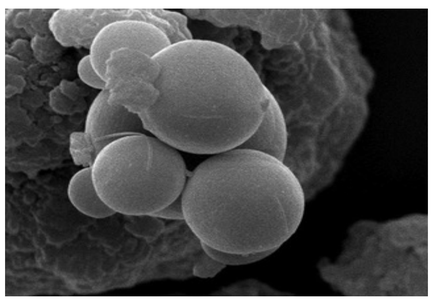
Aim

The cellular stress biomarkers (heat shock protein Hsp-70, p38 MAPK phosphorylation ratio, phosphorylated p38 MAPK/p38 MAPK, the antioxidant enzymes superoxide dismutase-SOD, catalase and glutathione reductase-GR) were investigated in the liver and muscle tissue samples of perch (*Perca fluviatilis*), to study differences in fish response under the treatment with virgin and aged/oxidized polyethylene MPs (PE-MPs).

Methodology

Specimens of the freshwater fish *Perca fluviatilis*, a commercially important fish species collected from Lake Volvi (northern Greece) were fed for 15 days with 100 μm, 1mg/g of food, of virgin polyethylene microplastics (PE-MPs), as well as with oxidized PE-MPs that were exposed to UV irradiation for 120 days (also referred as "aged" microplastics).

Perca fluviatilis (perch)



Polyethylene MPs

Results

FTIR spectra of PE-MPs revealed the appearance of hydroxyl/hydroxyperoxide groups after UV exposure (Fig. 1). Moreover, UV exposure had deteriorated the thermal stability performance of the studied samples and altered their morphology, making the surface of the particles progressively rougher, whereas pores and cracks were created. The parameters studied exhibited statistically significant elevated levels (1.5 to 4 fold increase) compared to the control, in both the investigated tissues (Figs. 2 & 3).

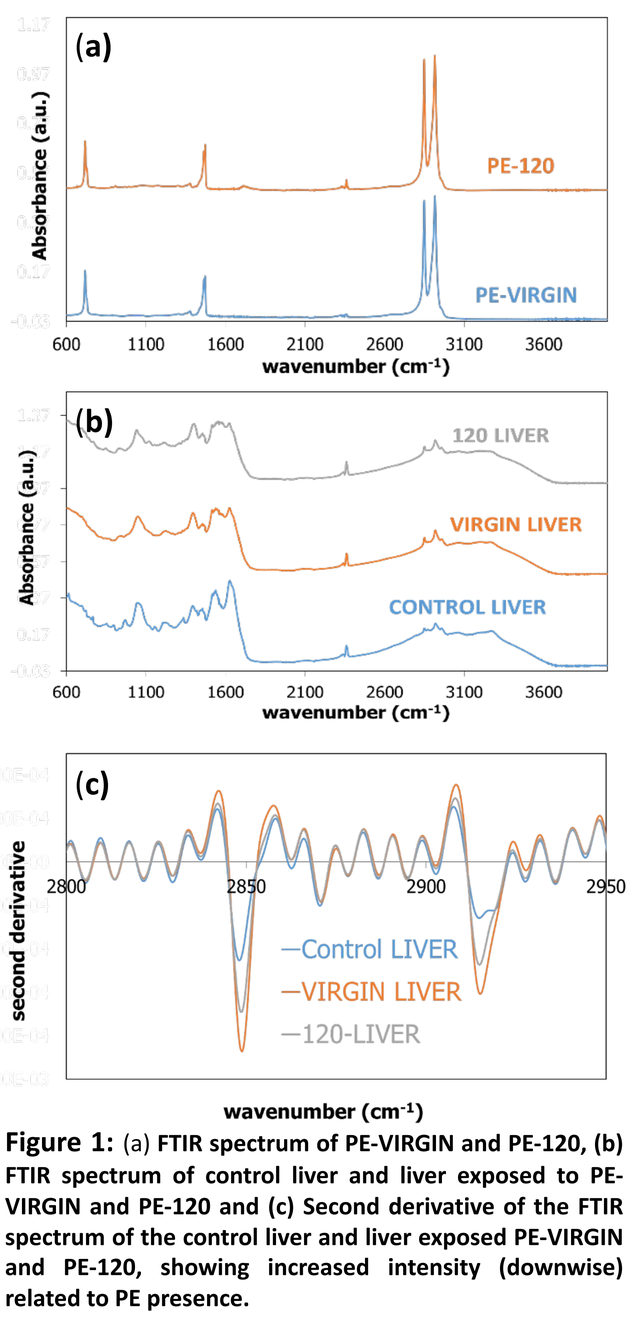


Figure 1: (a) FTIR spectrum of PE-VIRGIN and PE-120, (b) FTIR spectrum of control liver and liver exposed to PE-VIRGIN and PE-120 and (c) Second derivative of the FTIR spectrum of the control liver and liver exposed PE-VIRGIN and PE-120, showing increased intensity (downwise) related to PE presence.

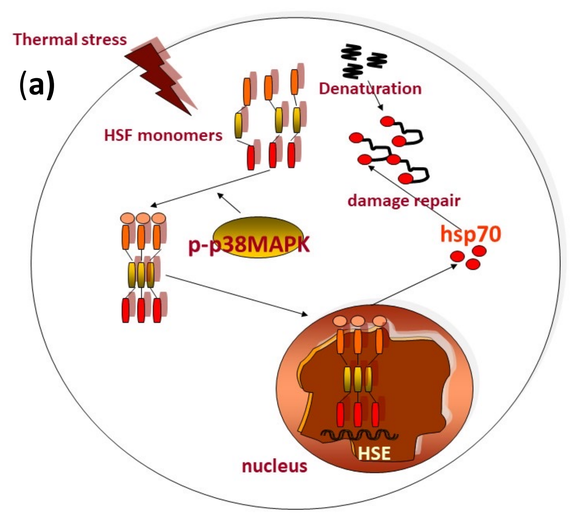


Figure 2(a): A model of the cellular responses elicited by MPs stress in *Perca fluviatilis*.

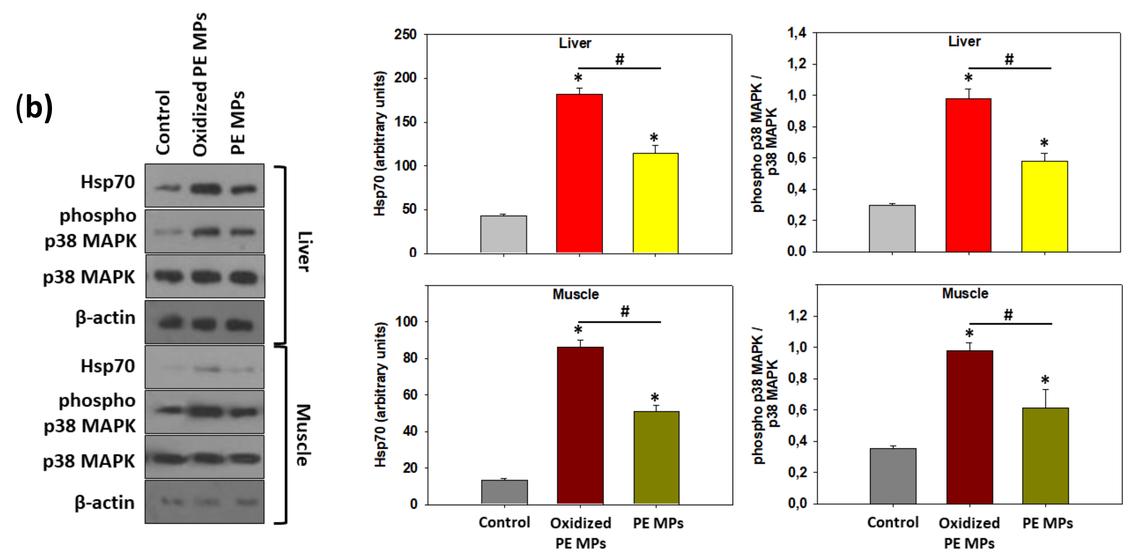


Figure 2(b): Hsp70 and p38 MAPK phosphorylation ratio levels in the liver and muscle of *Perca fluviatilis* exposed to PE-MPs and oxidized PE-MPs. Blots were quantified using scanning densitometry. Representative blots are shown. Mann-Whitney U test was employed to test for significance at $p<0.05$ between all experimental groups. *denotes significant differences ($p<0.05$) compared to the control group, while # denotes significant differences ($p<0.05$) between the two groups of PE-MPs.

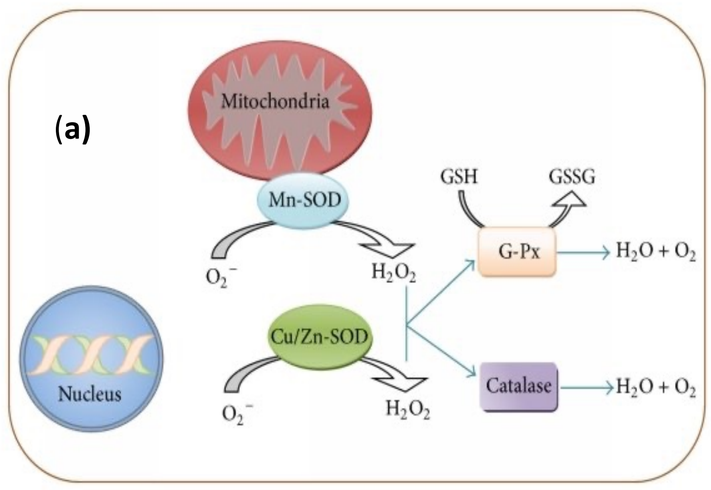


Figure 3(a): A model of the antioxidant defense elicited by MPs stress in *Perca fluviatilis*.

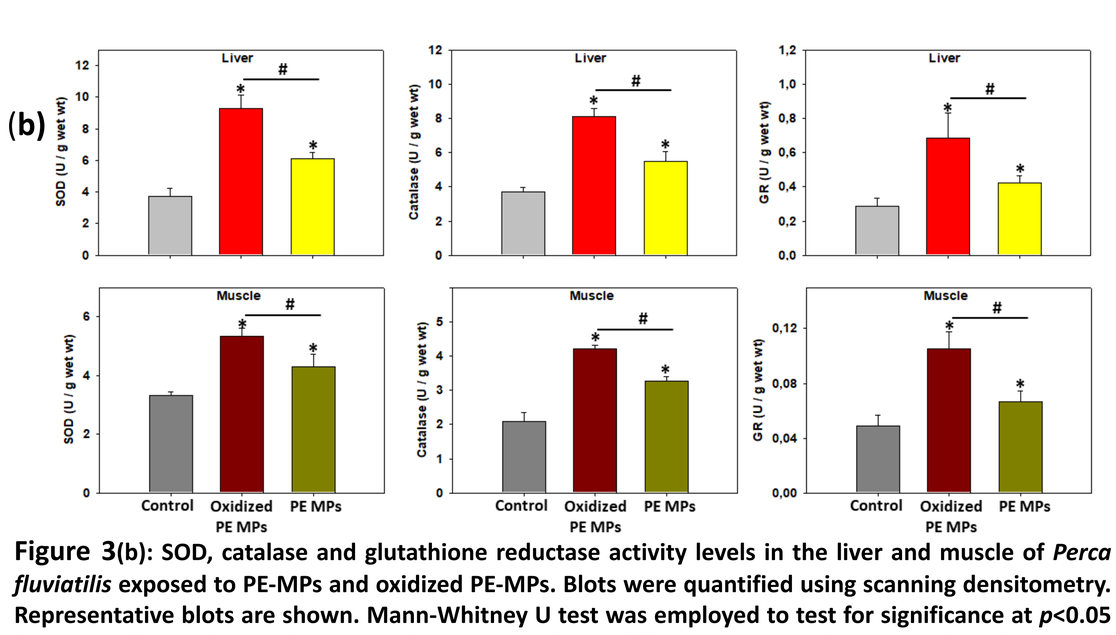


Figure 3(b): SOD, catalase and glutathione reductase activity levels in the liver and muscle of *Perca fluviatilis* exposed to PE-MPs and oxidized PE-MPs. Blots were quantified using scanning densitometry. Representative blots are shown. Mann-Whitney U test was employed to test for significance at $p<0.05$ between all experimental groups. *denotes significant differences ($p<0.05$) compared to the control group, while # denotes significant differences ($p<0.05$) between the two groups of PE-MPs.

Conclusions

Our results revealed that both virgin as well as oxidized, aged PE-MPs, at the concentration and duration of exposure tested, exert damage to fish tissues, as shown by the response of the parameters measured. It is worth mentioning that the harm the oxidized-aged microplastics exert on fish tissues is significantly higher than that of the same in size and concentration virgin microplastics. Therefore, the studied parameters may be suggested as biomarkers in biomonitoring studies against microplastics in aquatic ecosystems.

In addition, our results contribute to the elucidation of the mechanism induced by microplastics on tissues of aquatic organisms, since comprehending the magnitude of their impact on aquatic ecosystems is of great importance. Considering that fish consumption is only one of the routes of human exposure to microplastics, this study underlines the need for more research, risk assessment and implementation of measures to prevent human exposure to these particles.

References

[1] Bobori et al., *Journal of Molecular Sciences* (2022): 23, 13878; [2] Bobori et al., *Science of the Total Environment* (2022) 830: 154603; [3] Kaloyianni et al., *Toxics* (2021) 9: 289; [4] Dimitriadi et al., *Journal of Hazardous Materials* (2021) 416: 125969; [5] Kim et al., *Journal of Hazardous Materials* (2021), 413, 125423.