ASSESSMENT OF THE TOXIC EFFECT OF MICROPLASTICS AND METALS BY THE ACUTE TOXICITY TEST (*Daphnia magna*)

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INTRODUCTION

Plastic waste represents global contaminant due to constant growth of its production and use, but also inappropriate disposal. Microplastics (MPs) are particles smaller than 5 mm that, due to their small dimensions, easily reach all components of the environment, including organisms. According to UN, 30% of marine fish contained MP in their tissues, consequently causing gut blockages, reduced energy budgets or starvation and toxic effects [1]. Over the past decades, concerns about the potential of MPs as carriers of various pollutants (metals, organic contaminants, pharmaceutics) have raised intention, because their interaction can have multiple harmful consequences for organisms [2].

<u>GOAL</u>

Examination of the acute toxic effect of polystyrene (PS - 0.1-1500 μ m; 0.1-100 mg l⁻¹), different metals (Ag, Al, Co, Cu, Pb, Zn – 50 μ g l⁻¹ and Cd - 25-1000 μ g l⁻¹) and their combinations by testing toxic influence on the survival rate of the water flea (*Daphnia magna* Straus, 1820).



MATERIALS AND METHODS

The experiment was performed during 48 hours, including the following toxicity tests:

A/ effects of polystyrene (PS): influence of size (0.1-1500 μ m) and concentrations (0.1-100 mg l⁻¹); additionally, confirmation of PS ingestion in water flea was examined by stereomicrospcope and fluorescence microscopy;

B/ effects of different metals: Ag, Al, Co, Cu, Pb, $Zn - 50 \mu g l^{-1}$;

C/ effects of different Cd concentrations: 25, 50, 100, 200, 400, 600, 800, 1000 µg 1⁻¹;

D/ effects of the combination of Cd and PS: considering that MP were used at a size not accessible to water fleas (1.5 mm, 5 mg l^{-1}) in combination with Cd (50 µg l^{-1}), consequently binding of Cd to MP particles might remove metal from the medium, making Cd less available to water fleas and therefore expecting less toxicity compared to a test with the same concentration of Cd.

RESULTS

A/ The toxic effect of PS of 0.1 μ m; 1 μ m and 2 μ m size was 100%, of 3 μ m 80% and the lowest toxicity rate was recorded on "large" size particles, i.e. 1500 μ m with 20% effect. Regarding different concentrations, PS of 2 μ m had a maximum toxic effect of 60% at 5 mg l⁻¹, compared to 55% for the 80 μ m PS and 40% for the 1500 μ m PS, both at 100 mg l⁻¹. Microscopy techniques enabled visual confirmation of PS ingestion in the digestive truct of water flea;

B/ Toxicity of metals of the same concentration varied with metal tested and ranged 40-100% toxic effect; C/ Toxic effect of Cd increased with concentration: for 25 μ g l⁻¹ the toxicity was 40%, for 50 μ g l⁻¹ it was 60%, and 100-1000 μ g l⁻¹ Cd caused 100% toxicity;

D/ The combination of Cd (50 μ g l⁻¹) and PS (5 mg l⁻¹) of a size that is not accessible to the organism (1.5 mm) showed lower mortality (for 36%) compared to the organisms exposed to the same concentration of Cd , which preliminarily indicates the adsorption of Cd to MP.

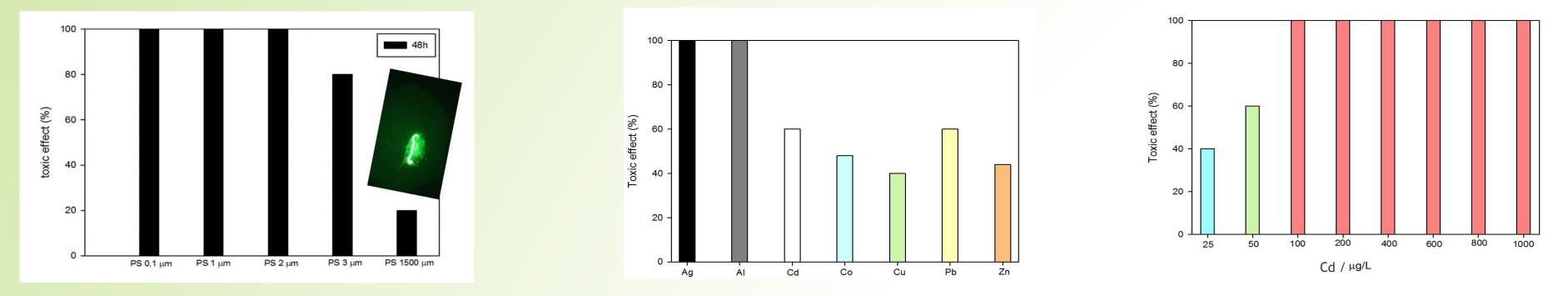


Phylum: Arthropoda Subphylum: Crustacea Class: Branchiopoda Family: Daphniidae











A/ Toxicity of PS depended on particle size and concentration, showing the highest toxicity for sizes in the range of the crustacean's food (20 nm-5 μ m), and increase in toxicity with concentration (EC₅₀ for 2 μ m = 3.48 mg l⁻¹, 80 μ m = 72.2 mg l⁻¹, 1500 μ m = 144 mg l⁻¹); The toxicity testing of MP should be carried with particle sizes that allow intake and exposure to the organism;

B/Toxicity of metals was specific, following the order Ag=Al>Cd=Pb>Co>Zn>Cu;

C/ Cd exposure confirmed increase of toxicity with concentrations, showing 100% mortality for Cd at concentrations 100-1000 μ g L⁻¹; D/ Preliminary results indicated possible binding of Cd to MP, and in the case when plastic is not available for ingestion in water fleatoxicity of Cd decreased, since Cd bonded to PS was not available for intake in water flea.

References

[1] C.M. Rochman, C. Brookson, J. Bikker, N. Djuric, A. Earn, K. Bucci, S. Athey, A. Huntington, H. McIlwraith, K. Munno, *Environ. Toxicol. Chem.* 38 (2019) 703.
[2] W. Yuan, Y. Zhou, Y. Chen, X. Liu, J. Wang, *Sci. Tot. Environ* 746 (2020) 141254.

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