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Effects of exposure to oxide nanoparticles (Al_2O_3 and ZnO) singly and mixtures on *Carassius auratus* gills

M. Benavides[*], P. Coelho[**], C. Lodeiro[***], M.S. Diniz[***]

*Departamento de Ciências e Engenharia do Ambiente. Faculdade de Ciências e Tecnologia. Universidade Nova de Lisboa. Campus da Caparica. 2829-516 CAPARICA, Portugal
[**]IMAR-CIC, Departamento de Ciências e Engenharia do Ambiente. Faculdade de Ciências e Tecnologia. Universidade Nova de Lisboa. Campus da Caparica. 2829-516 CAPARICA, Portugal
[***]REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Centro de Química Fina e Biotecnologia, Universidade Nova de Lisboa, 2829-516 Caparica.

e-mail: mesd@fct.unl.pt

Nanoparticles (NPs) are currently used in many industrial processes integrating several commercial products such as in biomedicine, cosmetics, ceramic to biomaterials and electronics among many others. They include several types of materials from metals, polymers, and have been defined as particles with at least one dimension in the order up to 100 nm [1]. For example, Zinc oxide NPs can be dispersed in industrial coatings to protect wood, plastics, and textiles from exposure to UV rays and Aluminium oxide NPs are used in cosmetics, electronics or as catalyst. In addition, the properties of diverse conventional materials change when formed from nanoparticles. This is typically because nanoparticles have a greater surface area per weight than larger particles which causes them to be more reactive to some other molecules and thus showing a high potential to cause toxicity. NPs are able to cross cell membranes and cause cell injury. Therefore, is very important to evaluate the toxicity of NPS, since their industrial production and uses will also result in large discharges to the aquatic environments via sewage effluents and cause damage to ecosystems [1,2,3].

The aim of the present work is to evaluate the toxicity of oxide NPs (Al and Zn) on gill gluthatione-S-transferase activity (GST), lipid peroxidation on the morphology of goldfish gills (*Carassius auratus*). Different suspensions of Al_2O_3 (~ 20 nm) and ZnO NPs (~ 50 nm), were prepared using distillate water and then ultrasonicated (10 min, 35 KHz). The suspensions were added to six 10L of previously de-chlorinated tap water in exposure tanks, to obtain nominal concentrations (10 Al_2O_3 and 10 ZnO NPs µg/L; 100 Al_2O_3 and 100 ZnO NPs µg/L; 10 Al_2O_3 + 10 ZnO NPs µg/L; 100 Al_2O_3 + 100 ZnO NPs µg/L). The test fish, *C. auratus* (n= 88), were randomly distributed by 6 exposure tanks and an additional tank with clean tap water was used as control. Fish were sampled following 7, 14, and 21 days of exposure. The enzymes activities were determined by following the procedure described by Habig *et al*. [4] for GST and lipid peroxidation was measured based on the *Thiobarbituric Acid Reactive Species* method [5]. The tissues were treated for histopathology according to Martoja and Martoja [6] and examined using a Leica-ATC 2000 microscope. In general, results show an increase of GST activities in gills tissues in comparison to controls, mainly following 14 days of exposure (Fig. 1), which was also supported by lipid-peroxidation data (MDA content). The results confirm that the specific properties and type of oxide NPs such as size, surface area, influenced the degree of toxicity observed. The results from histological observations (Fig. 2) showed that exposure to oxide NPs affected gill tissues, with changes being detected for both metal species which is in agreement with biochemical results. Morphological alterations include gill hyperplasia (with fusion of lamellae) as shown in Fig. 2a. The results suggest that oxide NPs provoke a resilient response to oxidative stress caused by exposure to oxide NPs.

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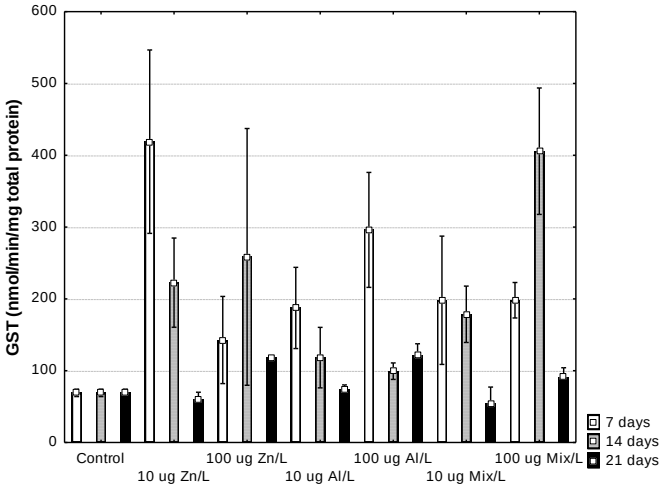

Figure 1. GST activity in C. auratus. GST total activity (mean±SD), measured in gills.

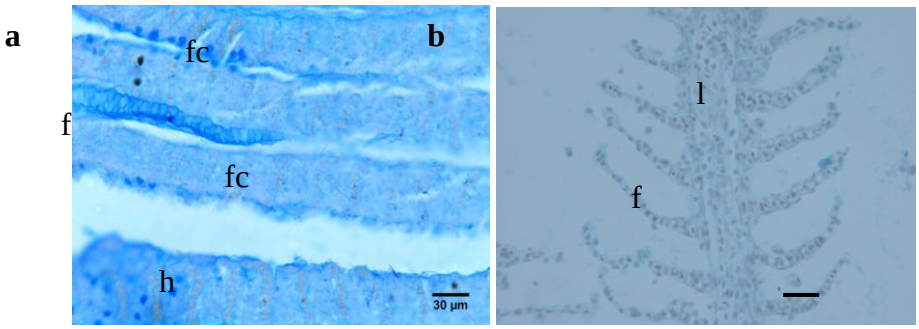

Figure 2. Representative image from fish gills exposed to (a) 100 Al_2O_3 + 100 ZnO NPs µg/L mixture and (b) control, after 21days of exposure. Legend: f, filament; l, lamellae; h, hyperplasia; fc, complete fusion of lamellae; (*) presence of mucous; Staining: Alcian Blue. Bar= 30 µm.

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