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Impact of silver nanoparticles on the biouptake, physiological responses and metabolic perturbations in freshwater alga *Poterioochromonas malhamensis*

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Given widespread use of silver nanoparticles (AgNPs), environmental risk assessment of these nanoparticles is of great importance. The toxicity of AgNPs was shown to be a result of an interplay of the particles uptake, dissolution and induction of the oxidative stress in aquatic organisms. Despite the progress in assessing their freshwater system implications, but little information exists concerning the metabolic perturbations induced by AgNPs on phytoplankton. Thus, the overall goal of the present study is to combine biouptake, physiological response and targeted metabolomics studies to elucidate metabolic perturbations in alga induced by AgNPs. We examine the interactions of citrate-coated 20 nm AgNPs compared with those induced by the dissolved Ag with the freshwater alga *Poterioochromonas malhamensis*. This golden alga often dominates mixotrophic phytoplankton population, and without cell wall can internalize these NPs.

Results of metabolomics evidenced that the exposure to AgNPs and dissolved Ag ions released by AgNPs resulted in time-dependent perturbation (exposure duration from 2 to 24h) of the concentration of metabolites involved in various metabolic pathways involving amino acids, nucleotides, fatty acids, TCA, antioxidants, photosynthesis and photorespiration. These perturbations of AgNPs toward *p. malhamensis* was attributed to the dissolved Ag ions. The exposure to AgNPs induced a significant accumulation of Ag in alga cell and increased with time. AgNPs internalized and accumulated in the vacuoles of *P. malhamensis* forming the agglomerates of 100 nm size. AgNPs internalized in food vacuoles contributed to the perturbation of amino acid metabolism, TCA cycle and oxidative stress. The physiological responses including ROS generation, lipid peroxidation, photosynthesis efficiency was also affected by AgNPs and dissolved Ag, particularly at 24 h exposure. These physiological responses confirm the metabolic perturbations observation. In this study, we demonstrate the integrated assessment of metabolomic and physiological changes for early detection of stress on phytoplankton induced by AgNP.

P19-06

Effect of PET and PVC microplastics on rainbow trout cell lines RTgill-W1, RTG-2 and RTL-W1

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The contamination of the aquatic environment by microplastics is an emerging issue requiring a thorough research of the potential effects microplastics can have on living organisms. One of the organisms commonly used in toxicological studies is the freshwater fish rainbow trout (*Oncorhynchus mykiss*). It has been shown that *in vitro* toxicity assays on rainbow trout cell lines are a suitable alternative to whole-fish assays, providing comparable results for many tested pollutants and offering a more ethical approach. Polyethylene terephthalate (PET) and polyvinyl chloride (PVC) are among the six most produced polymers, but their representation in toxicological studies is rather low. Therefore, the toxicity of PET and PVC particles was tested using three cell lines from rainbow trout, the RTgill-W1 (gill), RTL-W1 (liver) and RTG-2 (gonad) cell line. Multiple assays were applied to assess the changes in the metabolism of the exposed cells. The changes in cell viability were analyzed using three fluorescent dyes – alamarBlue, 5-carboxyfluorescein diacetate, acetoxymethyl ester and neutral red. In these assays, membrane integrity is evaluated as a marker of the general cell viability. The cells were also analyzed for changes in reactive oxygen species (ROS) generation and 7-ethoxyresorufin-*O*-deethylase (EROD) activity. The EROD assay enables to monitor the metabolism of xenobiotics by measuring cytochrome P450 1A induction. The ROS generation was studied using the 2´,7´-dichlorofluorescein diacetate assay. After the exposure to PET particles, no significant changes were observed. On the other hand, PVC exposure did induce a significant increase in ROS generation in all tested cell lines. When studying the source of this effect, chemical additives leaching from the plastics were identified as the major contributor. Due to the ongoing leaching process, the age of the tested suspensions also proved to influence the observed toxicity, suggesting that the assessment of this variable should not be omitted in future toxicological studies related to microplastics.

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P19-07

Ecotoxicity effects of $ZnCl_2$ and ZnO nanoparticles on *Daphnia magna* and *Tubifex tubifex*

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Toxicological properties of nanoparticles (NPs) are currently under investigation because of their large applications in industry and common-use products. Due to their well-known chemico-physical characteristics, ZnO NPs are among the most used NPs and consequently subjected to be dispersed in the environment. The chronic toxicity of ZnO nanoparticles is less well documented including some ZnO nanoparticle toxicity studies on soil species but studies on aquatic species are still very limited.

We focused on acute and reproductive effects of $ZnCl_2$ and ZnO NPs (<50 nm, <100 nm), in particular. Assessment of the impact of selected NPs on important representatives of the aquatic environment. The aim of this study was to investigate the chronic toxicity of the nanoparticles to *Daphnia magna* and *Tubifex tubifex*. Selected representatives are an important part of the fish food chain. The effect of the ZnO NPs and $ZnCL_2$ salt was tested on the growth and reproduction of *D. magna* in a chronic test scenario according to OECD guidelines 211. Tests with *Tubifex tubifex* were performed according to method ASTM E1706-04. The concentrations of $ZnCL_2$ and ZnO NPs tested were as follows: 0.01, 0.05, 0.1, 0.5, 1, 5, 10 $mg.l^{-1}$. Graphpad Prism was used for data visualization and statistics. Based on the chronic toxicity data, time to first brood, time between broods and number of broods per *D. magna* female were calculated. Dose-response curves were constructed and EC10,20,50 reproduction values were calculated.

Daphnia magna has been shown to be a better and more sensitive bioindicators than *Tubifex tubifex*. The unexposed daphnids started reproduction faster and had more broods per female than the ones exposed to the highest concentrations of nanoparticles and salts. It is obvious that the combined dissolution and aquatic species sensitivity distributions results indicate that the toxicity of these nanoparticles is mainly caused by dissolved metal ions. Based on the available information, no current risk of these nanoparticles to the aquatic environment is expected.

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