Molecular and cellular responses of mussels *Mytilus galloprovincialis* fed with the microalgae *Isochrysis galbana* exposed to PVP/PEI-coated silver nanoparticles at different seasons

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Bivalve mollusks have been identified as an important target group for nanoparticle (NP) toxicity because they are filter-feeding organisms able to uptake and process particles of different sizes. Several studies have been carried out in mussels exposed to NPs via water; however, there is scarce information on the effects of NPs ingested through the diet, especially at environmentally relevant concentrations. Further, the potential influence of season on mussel´s responses to NPs has not been explored. Silver NPs (Ag NPs) are being increasingly used due to their antimicrobial properties and therefore, concerns about their potential input and hazards in aquatic ecosystems are growing. Thus, with the aim of determining molecular and cellular responses to Ag NP exposure in mussels *Mytilus galloprovincialis*, dietary exposure experiments were performed both in autumn and in spring. Mussels were fed daily with the microalgae *Isochrysis galbana* previously exposed for 24 h to two different doses of PVP-PEI coated 5 nm Ag NPs: a dose of 1 µg Ag/L of Ag NPs close to estimated environmental levels and a higher dose of 10 µg/L Ag NPs. After 24 h of exposure, Ag concentration was measured in algae by ICP-MS while TEM and SEM analysis were performed in order to study NP fate. After 1, 7 and 21 days of mussel dietary exposure, total Ag concentration was measured in mussel soft tissues by ICP-MS and Ag deposits were measured at the light microscope after autometallography and localized by TEM followed by X-ray microanalysis. Microarray studies were performed to get a specific gene expression signature. Lysosomal membrane stability was measured in digestive cells as a general indicator of health status and genotoxic effects were assessed in hemocytes by Comet assay and the micronucleus test. Chemical analysis showed that microalgae exposed to 10 µg/L Ag NPs significantly accumulated Ag after 24 h. By TEM, electron dense deposits were observed between the scales and the membrane of microalgae and inside cells, indicating internalization of Ag NPs in algae. Mussels fed with exposed microalgae significantly accumulated Ag after 7 and 21 days in both seasons. Regarding genotoxic effects, DNA strand breaks increased significantly along the 21 days in spring and micronuclei frequency showed an increasing trend after 1 and 7 days of exposure to 1 µg/L Ag NPs in spring and to 10 µg/L in both seasons. Thus, PVP-PEI coated 5 nm Ag NPs were successfully transferred from algae to mussels and caused significant alterations in mussels.
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