Interaction of marine algae and nanoplastics, and the possible impact on the bioavailability of nanoplastics to primary consumers through the food-pathway

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Plastic pollution is a global problem. While macro- and microplastics are largely studied in terms of environmental concentrations and toxicity, a knowledge gap exists regarding nanoplastics (1-1000 nm diameter). Nanoplastics enter our environment both as primary products, engineered for cosmetic and pharmaceutical purposes, and as secondary degradation products. However, due to deficient sampling methods and analytical identification methods, information on environmental concentrations is lacking. Nevertheless, it is assumed that the exposure route, extent and rate of bio-uptake, and the nature of adverse effects will differ from those of microplastics, due to the reactivity features of nanoscale entities and the fact that they are small enough to cross biological barriers.

The aim of the study is to investigate the interaction between marine algae species and nanoplastics. We want to analyze whether there is an interaction, and what sort of interactions (aggregate formation, adsorption of the particles on the algal cell surface or absorption of the particles into the algae cell). Analysis of the quantity of particles that interact with algae is done using flow cytometry and visual inspection of the interactions is performed using fluorescence microscopy. Subsequently, we will analyze EPS (extracellular polymeric substances) production to investigate whether algae-aggregate formation is affected by the nanoplastics' presence. This is done using the Bradford assay, a colorimetric method for total protein determination. Data will be collected for two algae species and three different plastic types in three concentrations, every day during the exponential part of the growth curve, and every other day during the stationary phase of the growth curve.

The algae species used for the experiment are *Rhodomonas salina* and *Isochrysis galbana*, which are relevant algae species for the North Sea food web. Both species are exposed to concentrations of nanoplastics that are assumed to be environmentally realistic, using data on micro-plastic concentrations from measurements in the Port of Ostend.The nanoplastics used are fragmented hydrophilic PET, PP and PE, with a broad unimodal size distribution: diameters in the range from 40 nm to 5 µm. For PET, 90% of the particles have a diameter smaller than 1 µm, and for PP, 90% of the particles have a diameter smaller than 2.9 µm.

As it is yet unknown how fast and to what extent microparticles will degrade in nature, the calculation of the factor to convert observed microplastic concentrations to assumed nanoplastics concentrations is based on mass conservation principles. The factor is determined for the fragmentation of spherical microparticles with an average diameter of 250 µm to spherical particles with the size distribution of the plastic dispersions present in the lab. The starting point of 250 µm is based on measurement data in the Port of Ostend.

This research will be valuable for studying the effect of algae-NP interaction and provides relevant information on algae as a potential exposure pathway for marine copepod species such as *Nitocra spinipes* and *Acartia tonsa*.

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