



PLASTISPHERE ANALYSIS IN LAKES: MICROBIAL DIVERSITY, POTENTIAL IMPLICATIONS FOR FRESHWATER ECOSYSTEMS AND PUBLIC HEALTH AND POTENTIALITY IN PLASTIC POLYMERS BIODEGRADATION

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Plastic and microplastics (MPs) are considered an emerging pollutant in aquatic ecosystems. Although several studies focused on the effects of MPs on animal and plant life, the effects of MPs as vehicle for microbial dispersal among environments remain unclear. Plastic debris and MPs provide a durable surface that can be colonized by planktonic microorganisms and transported for long distances, supporting the growth of microbial biofilms. Such new ecosystem is referred to as the 'plastisphere' and numerous studies have demonstrated that the plastic-associated microbial communities differ in structure and composition from those in the surrounding waters. Plastisphere communities can play a significant role in transportation of potential invasive and pathogenic species and/or of bacteria with antibiotic-resistant genes (ARGs), facilitating pathogenicity and antibiotic resistance transfer to the environment, with ultimate risk for ecosystem and human health. AR genes and/or pathogenic species associated to MPs have been scarcely monitored yet and further research is needed to assess their impact on ecosystem and human health. Although recent investigations revealed a massive presence of MPs in rivers, lakes, and reservoirs, little is known about plastisphere in freshwater ecosystems. Moreover, MPs and plastics have shown to provide substrata that support the growth of microorganisms potentially capable of degrading plastic polymers. Lot of reports described the partial degradation of different plastic types by several microbial strains, but investigations are still required to elucidate the role and the extent of plastisphere contribution to plastic biodegradation processes. Thus, the present PhD project aims i) to assess the presence of ARGs and potential pathogenic bacteria in MPs-attached biofilms sampled in several Italian lakes during 4 yearly campaigns (Goletta dei Laghi); ii) to evaluate the diversity, the structure and the biodegradative potential of the plastisphere in microcosm experiments using lake water as biofilm source community. Droplet Digital PCR will be used to identify and quantify ARGs encoding resistance to different classes of antibiotics (tetracyclines, quinolones, sulphonamides, beta-lactamases, macrolides). Loop-Mediated Isothermal Amplification (LAMP-PCR) will allow the detection of biofilm-forming opportunistic pathogens (*Legionella pneumophila* and *Pseudomonas aeruginosa*). PET, MaterBi, and PLA (obtained from virgin polymers, common products, and UV pre-treated polymers) will be employed as substrata for microbial community adhesion and development. High throughput sequencing of 16SrRNA and 18SrRNA genes and Fluorescent *In Situ* Hybridization, coupled with catalysed reporter deposition (FISH-CARD), will allow to assess biodiversity and structure of bacterial plastisphere as a function of polymer types and biofilm age under confocal laser scanning microscopy (CLSM). In addition, Fourier Transform Infrared Spectroscopy (FT-IR), in Attenuated Total Reflectance mode (ATR), Water Contact Angle measurement (WCA), and Scanning Electron Microscopy (SEM) will be used to evaluate structural changes in the selected plastic substrata attributable to the plastisphere biodegradation activity over the microcosm experiment periods.