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Conference Abstract

A new and straightforward protocol for microplastic's DNA extraction from freshwater environments

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Abstract

Microplastics (< 5 mm particles) are ubiquitous environmental contaminants, capable of providing an artificial substrate for the development of microbial aggregates distinguished between water column, seston, and sediments distinct in taxonomic composition from those on natural surfaces such as water column, seston, and sediment. These complex communities, constituted by prokaryotic and eukaryotic microorganisms, represent a peculiar micro-ecosystem, identified only recently as "plastisphere". The microbial composition of the plastisphere associated with microplastics in surface waters is poorly known. Therefore, there is a need for a thorough investigation into the possible role of microplastics as a transport vector for biological contaminants of hygienic-sanitary interest. Several studies have described molecular techniques used to extract nucleic acids from aquatic environments and environmental matrices. However, little is known about the DNA extraction methods used for plastic. This study's main objective is to develop a protocol for microplastic's DNA extraction, improving the genetic material’s quantity and quality necessary for future applications such as gene amplification and sequencing. DNA was extracted from microplastic and environmental samples of a freshwater ecosystem. Two different microplastics were analyzed: a) environmental particles collected from an Italian river (Ofanto river) composed prevalently by black and transparent fragments of 5-1mm in

size, b) virgin microplastics purchased by local industry used as controls and composed by PE transparent pellets of 5mm and green PE particles of 100µm. DNA extraction was also performed from superficial river water, suspended organic matter (OM), and sediment of the river taking care to process three replicates for each type. Starting from the commercial DNeasy PowerSoil Kit, the method was optimized testing the minimum microplastic's amount, incubation parameters, time of cell lysis, and final elution volumes. We collected material with forceps from the glass containers for the environmental microplastics and OM, placing about 250mg of each replicate into 2-mL microcentrifuge tubes. We separated the 5 L water samples into three 500 mL aliquots and filtered each one with Whatman 0.2-µm filters. The filters were cut with sterilized forceps and placed into 2-mL microcentrifuge tubes (Debeljak et al., 2017). The main modifications made to the extractive protocol have been a method alternative of cell lysis including a preliminary incubation at 60 °C for 20 minutes and a reduction of the final elution volume to 70 µl in order to concentrate the extracted DNA. The quantity and quality of the extracted DNA were obtained respectively through the use of Qubit™ dsDNA HS Assay Kit (ThermoFisher), and agarose gel electrophoresis. Our results showed that the minimum amount of microplastics useful to obtain a valid genomic extract is about 120 mg. The modifies applied to the commercial kit resulted in significantly improving the material extracted more than eight times. The nucleic acids extracted from environmental microplastics applying the improved protocol ranged from 7.97 to 14.1 ng μl^{-1} with a mean value of 10.5 ± 3.2 ng μl^{-1} respect a medium value of 1 ± 0.1 ng μl^{-1} of material extracted from the same samples using the unmodified protocol of the commercial kit. As expected, the virgin microplastic samples did not show a measurable amount of DNA (< 0.2 ng μl^{-1}). Concentrations of DNA extracted from aqueous samples resulted in a mean value of 19.53 ± 6.47 ng μl^{-1}, while the amount of genetic material attached to sediment and OM samples revealed a concentration of 35.1 ± 2.19ng μl^{-1} and a value above the detection limit of 100 ng μl^{-1} respectively.

Keywords

microplastics, DNA extraction, methodologies, freshwater environments

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