



# Evidence on the effectiveness of mosses for biomonitoring of microplastics in fresh water environment

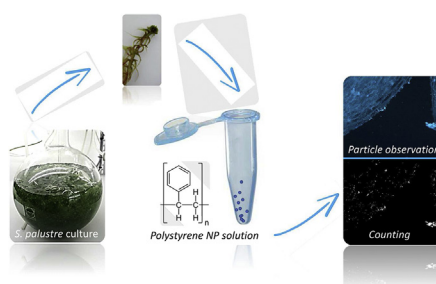
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## HIGHLIGHTS

- The ability of *S. palustre* to intercept and retain polystyrene NPs was tested.
- *S. palustre* retained NP aggregates more in devitalized than in living moss.
- NP entrapped increased while increasing the exposure time.
- Post-exposure washing induced a loss of larger aggregates.
- Particle count showed that small aggregates were the most abundant.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Mosses are well known as biomonitors of fresh water for metal pollutants, but no studies were reported so far about their ability to intercept plastic particles, although this kind of pollution has become an urgent issue worldwide. In the present work, the interaction between the moss *Sphagnum palustre* L. cultured in vitro and polystyrene nanoparticles (NPs) was studied for the first time in a laboratory experiment, in the view of using moss transplants for detecting microplastics in fresh water environments. The ability of *S. palustre* to intercept and retain polystyrene, and the effects of vitality and post-exposure washing on NP retention by moss were tested. Fluorescence microscope observations showed that polystyrene NPs were retained by moss leaves in form of small (the most abundant fraction) and large aggregates. Particle count analysis highlighted that the number of particles increased while increasing the exposure time. Moreover, moss devitalization favored NP accumulation, likely because of cell membrane damages occurred in dead moss material. Post-exposure washing induced a loss of larger aggregates, suggesting that exposure time is a key point to be carefully evaluated in field conditions. These results encourage the use of *S. palustre* transplants for monitoring microplastics contamination of fresh water environments.

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## 1. Introduction

Although mosses are especially used as effective bioaccumulators of airborne inorganic and organic pollutants, mainly

metals, metalloids and polycyclic aromatic hydrocarbons (Capozzi et al., 2017; Di Palma et al., 2017; Iodice et al., 2016), they proved to be efficient bioaccumulators of trace elements when transplanted in fresh water systems, irrespectively of their vitality (Cesa et al., 2006, 2009, 2010; Debén et al., 2016). This capacity is strictly related to their high surface to mass ratio, to specific surface properties and characteristics of the cell wall and to the ability to

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entrap particle bound pollutants in their tissues (González and Pokrovsky, 2014). The lack of a cuticle on leaflets and stem makes the cell wall an overall hydrophilic compartment, i.e. capable to absorb water, ions and small molecules, and this is a key mechanism ensuring moss mineral nutrition. Moreover, adsorption and absorption are favored by the presence of numerous functional groups on the cell wall; for example, a specific surface area of  $28 \pm 1 \text{ m}^2 \text{ g}^{-1}$  d. w., with  $0.65 \text{ mmol g}^{-1}$  proton/cation binding sites (mainly carboxylic and phenolic groups) was found in a clone of *Sphagnum palustre* selected among several moss species because it is the most inert and efficient metal and proton adsorbent (González and Pokrovsky, 2014; Gonzalez et al., 2016). All the above mentioned traits favor the interception and retention of particulate matter of a wide range of dimensions onto moss surface; PM (including the hazardous ultrafine fraction) represents in fact the main form in which pollutants are intercepted and accumulated by these plants (Di Palma et al., 2017).

In the recent years, technological advancement has led to an increased production and release into the environment of a huge variety of nanometer-sized particles with a wide assortment of chemical composition. Nanoparticles are currently employed in numerous industrial processes (e.g., Ag nanoparticles with antibacterial and antifungal properties used as additives in air sanitizer sprays, detergents and toothpaste; functionalized fullerenes -carbon empty sphere-incorporated into cosmetics, for delivering skin nutrients) contaminating all major environmental compartments at a global scale (e.g. Piccinno et al., 2012).

The toxicity of nanoparticles is specifically related to their minute size, smaller than cells and cell organelles, allowing them to penetrate these fundamental biological structures, and altering their normal functions; examples of toxic effects induced by nanomaterials include tissue inflammation, increase of oxidation processes, up to cell death (Anderson et al., 2016; Forte et al., 2016; Buzea et al., 2007), even if for some of them, like cerium and yttrium oxides, a beneficial effect has been recognized (Schubert et al., 2006).

While a considerable number of papers exists dealing with metal accumulation in mosses (e.g. Boquete et al., 2016; Di Palma et al., 2017; Harmens et al., 2015), a limited number is addressed to organic pollutants; among these latter, mosses could accumulate PAHs, particularly those with high molecular weight and associated to PM (Capozzi et al., 2017; De Nicola et al., 2013a, 2013b; Spagnuolo et al., 2017) and dioxins (Carballeira et al., 2006).

Among the emerging organic pollutants, at present, plastics hold a dominant position due to their worldwide diffusion and long residence time in the environment; the scientific community is giving plastics increasing attention by studying their degradation processes and effects on ecosystem health (Yoshida et al., 2016). The term “plastic” indicates a family of organic polymers derived from petroleum sources, including polyvinylchloride (PVC), nylon, polyethylene (PE and PET), polystyrene (PS), and polypropylene (PP) (Vert et al., 2012). In general, microplastics fall into two categories: primary microplastics, produced intentionally (e.g., microbeads, plastic pellets) and secondary microplastics deriving by degradation of larger plastic to smaller pieces (e.g. Cole et al., 2011; Andersson, 2014). Whatever their origin, about 300 million tons of plastics have been produced from the 1950 s to 2014 (Plastic Europe, 2015), and their fate is to accumulate within the oceans worldwide (Browne et al., 2011).

Microplastics and nanoplastics pose aquatic environments at risk because of their ubiquity, the long residence times, and tendency to be ingested by living organisms (Arthur et al., 2008; Galgani et al., 2010; Andrady, 2011). Among plastics, polystyrene is one of the most abundant polymers discarded into rivers and marine environment (Hidalgo-Ruz et al., 2012); small pieces

(40–500 nm) are recognized to have the most toxic effects (Lee et al., 2013; Varela et al., 2012). For example, Tussellino et al. (2015), tested NPs of polystyrene at a concentration of 18 mg/L founding an embryotoxic effect on the model organisms *Xenopus laevis*. Microplastics have been isolated in sediments, water volumes and water surface; size, buoyancy and density of the plastic polymers have been mainly used as analytical tools for their characterization, after or not a concentration step. To avoid misidentification of small particles that can be confused with organic matter some approaches have been attempted as enzymatic digestion, or electrostatic separation, when it was not possible to use more accurate methods as Fourier transform infrared spectroscopy (FT-IR) (Hidalgo-Ruz et al., 2012). However, the very small particles, those with a nano-size, can be overlooked; thus, the possibility of detecting microplastics in waterbodies by a biomonitor could represent a first qualitative approach, before systematic sediment/water collection step.

For all the above considerations and based on the high ability of mosses to accumulate and retain PM pollutants, the interaction between the moss *Sphagnum palustre* and polystyrene nanoparticles was studied in a laboratory experiment aimed at investigating: i) the ability of the moss to intercept and entrap polystyrene nanoparticles; ii) the influence of moss vitality on the accumulation; iii) the effect of an extensive post-treatment washing on polystyrene retention. In particular, we have used uncoated fluorescent polystyrene nanoparticles (50 nm) because the maximal cellular uptake of NPs can take place in the size range of 40–60 nm and fluorescence offers an efficient tracking method (Nam et al., 2013). We have employed a concentration in the range already tested in other organisms (Tussellino et al., 2015; Casado et al., 2013). This experiment was intended as a laboratory trial in the view of using moss transplants for detecting microplastics in fresh water environments for biomonitoring purposes.

## 2. Material and methods

### 2.1. Plant material

A clone of the moss *Sphagnum palustre* L. cultured in vitro was used in the experiments; this clone was previously selected among several moss species for its best performance in PM interception and retention (González and Pokrovsky, 2014; Gonzalez et al., 2016).

### 2.2. Polystyrene NP characterization

We purchased red fluorescent unmodified polystyrene NPs, with diameters of about 50 nm, from Duke Scientific Corporation. Composition: Polystyrene. Dyes: Firefli fluorescent red (542/612 nm). Density:  $1.05 \text{ g/cm}^3$  ( $1.5 \times 10^{14}$  NPs/ml). Index of Refraction: 1.59 at 589 nm (25 °C).

Dynamic light scattering (DLS), made with a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK), was performed to measure polystyrene NPs size and z-potential. Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. The zeta potential is a key indicator of the stability of colloidal dispersions. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in a dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability, i.e., the solution or dispersion will resist aggregation. When the potential is low, attractive forces may exceed this repulsion and the dispersion may break and flocculate. So, colloids with high zeta potential (negative or positive) are electrically stabilized while colloids with low zeta

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