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Silver nanoparticles uptake by salt marsh plants – Implications for phytoremediation processes and effects in microbial community dynamics

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ABSTRACT

This study investigated the uptake of silver nanoparticles (AgNPs) by a salt marsh plant, *Phragmites australis*, as well as AgNPs effects on rhizospheric microbial community, evaluating the implications for phytoremediation processes. Experiments were carried out with elutriate solution doped with Ag, either in ionic form or in NP form. Metal uptake was evaluated in plant tissues, elutriate solutions and sediments (by AAS) and microbial community was characterized in terms of bacterial community structure (evaluated by ARISA). Results showed Ag accumulation but only in plant belowground tissues and only in the absence of rhizosediment, the presence of sediment reducing Ag availability. But in plant roots Ag accumulation was higher when Ag was in NP form. Multivariate analysis of ARISA profiles showed significant effect of the absence/presence of Ag either in ionic or NP form on microbial community structure, although without significant differences among bacterial richness and diversity. Overall, *P. australis* can be useful for phytoremediation of medium contaminated with Ag, including with AgNPs. However, the presence of Ag in either forms affected the microbial community structure, which may cause disturbances in ecosystems function and compromise phytoremediation processes. Such considerations need to be address regarding environmental management strategies applied to the very important estuarine areas.

Capsule: The form in which the metal was added affected metal uptake by *Phragmites australis* and rhizosediment microbial community structure, which can affect phytoremediation.

1. Introduction

Nanotechnology has grown very quickly in the past few years and manufactured nanoparticles (NPs) have been broadly used in medicine, health care, biomedical products, pharmaceuticals, children toys, washing machine coatings, electronic devices, engineering materials, wall paints, water purification, textiles, personal care products, renewable energies, transportation, agriculture (fertilizers and plant protection products), fishing, environmental remediation, kitchen – ware, manufacturing, cosmetics, wound dressing, food packaging and security (Fabrega et al., 2011; Mohanty et al., 2014; Shah et al., 2014 and references therein; Burić et al., 2015; Gambardella et al., 2015; Klitzke et al., 2014).

Silver nanoparticles (AgNPs) are one of the most used NPs with production volumes of 500 t per year (Burić et al., 2015). These NPs can end up in the environment directly, through sewage discharges (released from household and industrial products into wastewaters), run-off and aerial deposition, and indirectly through organic fertilizers and plant production products (deposition into soils and water), atmospheric emissions and accidental spills during the manufacturing and transport (Fabrega et al., 2011; Baker et al., 2014; Klitzke et al., 2014; Nam et al., 2014).

The toxicity of AgNPs and their implications in the environment have been intensively studied since these NPs are, among all, the most likely to produce an effect in ecosystems (Ma et al., 2015a), having a cumulative impact in the environment and in human health (Klitzke et al., 2014). Actually, AgNPs are classified as extremely toxic according to the L(E)C50 values (the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals) for environmentally relevant organisms (L(E)C50 < 0.1 mg L^{-1}) (Burić et al., 2015) and predicted environmental concentrations (PECs) for AgNPs in the environment range between 0.03 and 0.08 mg L^{-1}. In addition, a strong antibacterial potential of AgNPs have been reported by several authors (Braun et al., 2015; Gambardella et al., 2015; Kumahor et al., 2015; Metreveli et al., 2015). Despite all this, there is still a lack of knowledge about the consequences that NPs can inflict to human health and to ecosystems (Kühn et al., 2014 and references therein).

AgNPs can be very stable throughout their path to aquatic systems due to their ability to sorb onto dissolved organic matter. Consequently,

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they can reach estuaries and marine ecosystems as final sinks (Burić et al., 2015).

Phytoremediation, a technology based in natural processes using plants for removal, accumulation, attenuation, degradation, absorption or metabolization of inorganic and organic pollutants from contaminated sites (soil, water and air) (Fulekar, 2012; Evangelou et al., 2015; Khan and Bano, 2015; Padmapriya et al., 2015), presents a sustainable solution for the recovery of impacted ecosystems, such as estuarine areas. Estuaries are highly productive ecosystems but, at the same time, they are very sensitive and fragile (Bouvy et al., 2010). They have been suffering a high anthropogenic pressure receiving all type of contaminants, through point or diffuse sources, being imperative new remediation and recovery strategies.

There is a sort of advantages in the application of phytoremediation such as low cost, improvement of soil and water quality, high rhizosphere activity stimulation and negligible site disruption (Khan and Bano, 2015; Padmapriya et al., 2015). However, metal phytoremediation can be negatively affected if the metal takes the NP form (Andreotti et al., 2015).

Nanoparticles can suffer several physical – chemical and colloidal transformations being the most important aggregation and disaggregation, sorption onto natural organic matter and dissolution (Metreveli et al., 2015; Schaumann et al., 2015). These transformations can influence the bioavailability, uptake, accumulation and stability of metallic NPs, as well as their toxicological effects (Burić et al., 2015; Farkas et al., 2015; Gambardella et al., 2015). In fact, NPs toxicity to plants and associated microorganisms can represent an obstacle to an effective remediation (Johnson et al., 2004; Xu et al., 2015). Rhizosphere microorganisms can be key players in plants phytoremediation potential. But, bacterial communities are sensitive to external pollutants and shifts in the community composition and productivity can be compromised, affecting the functioning of ecosystems (Farkas et al., 2015 and references therein) and ecosystem services, since microbial communities have a key role in decomposition and mineralisation, inorganic nutrient cycling, disease causation and suppression, and pollutant removal (Bissett et al., 2013).

Considering all the exposed one can say that increased use of NPs, including AgNPs, raises the probability of these NPs to reach the environment, including estuaries. Although a lot of information exists regarding the interaction of plants with metals there is a lack of information among the interaction of metallic NPs with plants, including with AgNPs and particularly for saltmarsh plants. Phytoremediation is a cost-effective technique that can be applied to clean and recover moderately impacted areas as estuarine areas. Most salt marsh plants are able to immobilize and store metals in sediments surrounding their roots and in belowground and aboveground tissues, showing potential to phytoremediate metals contaminated sediments which can be a valuable tool for the management of the very sensitive and ecological important estuaries areas. So, to test if the phytoremediation potential of salt marsh plants is not altered when metals take the form of NPs is of extreme importance. Moreover, microorganisms associated to plant roots rhizobacteria can have a significant role on the phytoremediation potential of salt marsh plants. Since AgNPs can be toxic to bacterial communities, evaluating if salt marsh rhizobacteria are affected by the presence of engineering NPs is also necessary, because it can affect phytoremediation processes.

The aim of this work was to investigate, under controlled conditions, the uptake of AgNPs by *Phragmites australis*, a salt marsh plant, evaluating the implications for phytoremediation processes, as well as AgNPs effects in the microbial community of sediments associated with the plant roots (rhizosediment). Plants were exposed to elutriate prepared with their rhizosediments and nearby estuarine water, a simplified but environmentally relevant medium, doped with Ag, either in ionic or in NP form. Salt marsh plants such as *P. australis* have shown potential to be used in phytoremediation processes in estuaries to treat metal contaminated sediments (Nunes da Silva et al., 2014). This work hypothesises that salt marsh plants may also have a role in the metal NPs remediation in estuarine areas.

2. Material and methods

2.1. Sampling site

Phragmites australis with the respective rhizosediment (sediment in contact with plant roots, cubes with ca. 10 cm × 10 cm × 10 cm) and water nearby were collected from Lima river estuary in North of Portugal (41.418N; 08.518W (WGS84)), at low tide, in May of 2014. Plants without a senescent appearance, with similar size and age, were collected. In the laboratory, rhizosediment was separated from the roots, homogenized and set aside for the experiments, as described in Section 2.2. A portion of this rhizosediment (initial sediment) was stored at −20 °C for posterior microbial analysis and another portion was put to dry at room temperature for metal assessment. Plants were washed with deionized water (to remove all traces of sediment) and kept in a nutrient solution (one quarter strength modified Hoagland nutrient solution (Hoagland and Arnon (1950)) until the beginning of the experiment (Andreotti et al., 2015).

2.2. Microcosms assembly

Elutriates were prepared accordingly to Environmental Protection Agency protocols (USEPA, 1991, EPA 503/8-91/001), also described in previous work (Fernandes et al., 2015a). For that, per flask, 50 g of rhizosediment were mixed with 200 mL of estuarine water and manually shaken to break soil clods. After that, all flaks were placed on a shaker for 30 min, at room temperature, in a reciprocal movement (at 100 rpm) and were left to settle for 24 h before the beginning of the experiments, at room temperature. Two experiments were assembled, one just with elutriate solution and another with rhizosediment soaked in the respective elutriate. For the first experiment, elutriate solutions were decanted and filtrated through 0.45 μm pore size filters (cellulose nitrate membrane, Millipore), to eliminate particulate suspended matter (except colloids), homogenized and distributed through new flasks. For the second experiment flasks were used as prepared after settling.

For each experiment, 3 treatments (all in triplicate) were tested: control (without added Ag (C)); addition of Ag in ionic form to obtained a 10 mg L^{-1} Ag concentration in solution (Ag (I) in ionic form, $AgNO_3$ p.a. from Merck); and addition of 2.0 mg of AgNPs (Ag in NP form, Ag (I) oxide, nanopowder, <100 nm particles size containing PVP as a dispersant (as indicated by the manufacture Aldrich)). The complete dissolution of the NPs would result in the same Ag concentration (10 mg L^{-1}) in the elutriate solution and, therefore, total Ag concentrations were the same for both cases.

These metal concentrations were selected based on studies carried out for other metals (e.g. Cu) (Almeida et al., 2008; Rocha et al., 2014; Andreotti et al., 2015) and although higher than the respective effect rang – median (ERM) and unlikely to be found in the environment, they may cause a more pronounced effect on plants, producing measurable results in short term experiments. ERM is the sediment quality guideline that indicates the pollutant concentration above which adverse biological effects may frequently occur in marine and estuarine sediments (Long et al., 1995).

To half of the flasks of each treatment doped with Ag, *P. australis*, plants were added (3 plants per flask). Plants' roots were previously washed for 1 min with sodium hypochlorite solution (8%, v/v) followed by 30 s with deionized water, to remove all microorganisms in plants' roots that could interfere with the final results. The other half of the flasks were left without plants. For the control treatment, only systems without plants were prepared. Even in such a short time exposure experiment, plants can affect microbial community dynamics (Fernandes et al., 2015a) being a confounding factor for data interpretation.

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