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Response of microbial communities colonizing salt marsh plants rhizosphere to copper oxide nanoparticles contamination and its implications for phytoremediation processes

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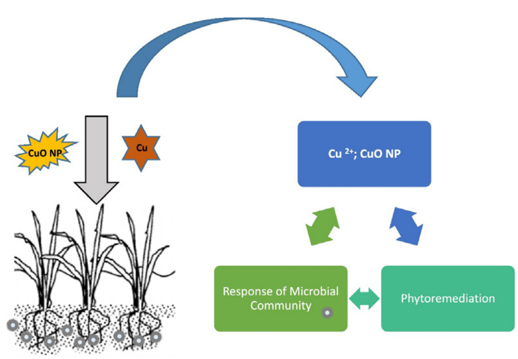
HIGHLIGHTS

- Metal presence and metal form changed rhizosediments microbial community structure.
- Metal uptake in plant roots lower or not significant when metal nanoparticles added.
- CuO nanoparticles increased *Phragmites australis* rhizosediment microbial abundance.
- Nanoparticles decreased *Halimione portulacoides* rhizosediment microbial abundance.
- Nanoparticles shifts in community structure may alter ecosystems functions.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aimed to investigate Cu oxide nanoparticles (CuO NP) effect on microbial communities associated with salt marsh plants (*Halimione portulacoides* and *Pragmites australis*) rhizosphere and its implications for phytoremediation processes. Experiments were conducted, under controlled conditions, over one week. Rhizosediment soaked in the respective elutriate (a simplified natural medium) with or without plants, was doped with CuO NP or with Cu in ionic form. Microbial community in rhizosediments was characterized in terms of abundance (by DAPI) and structure (by ARISA). Metal uptake by plants was evaluated by measuring Cu in plant tissues (by atomic absorption spectroscopy). Results revealed significant metal uptake but only in plant roots, which was significantly lower (*H. portulacoides*) or not significant (*P. australis*) when the metal was in NP form. Microbial community structure was significantly changed by the treatment (absence/presence of Cu, ionic Cu or CuO NP) as showed by multivariate analysis of ARISA profiles and confirmed by analysis of similarities (Global test - one way ANOSIM). Moreover, in *P. australis* rhizosediments microbial abundance, bacterial richness and diversity indexes were significantly affected (increased or decreased) due to metal presence whereas in *H. portulacoides* rhizosediment microbial abundance showed a significant decrease, particularly when the metal was in NP form. Accordingly, Cu presence affected the response of the rhizosphere microbial community and in some cases that response was significantly different when Cu was in NP form. The response of the

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microbial communities to Cu NP might also contribute to the lower metal accumulation by plants when the metal was in this form. So, Cu NP may cause disturbances in ecosystems functions, ultimately affecting phytoremediation processes. These facts should be considered regarding the use of appropriate salt marshes plants to remediate moderately impacted areas such as estuaries, where NPs can be found.

1. Introduction

Development of nanotechnology has grown in the past few years (Nogueira et al., 2012) and manufactured metal oxide nanoparticles (NPs) has been used for medical, agricultural and industrial applications (Xu et al., 2015 and references therein). Copper oxide NPs (CuO NP) are one of the NPs most used in nanotechnology. These NPs are commonly used in gas sensors, catalysts, ceramic pigments, photovoltaic cells and semiconductor devices (Jiang et al., 2002; Godwin et al., 2009; Baek and An, 2011 and references therein).

The extensive use of engineered nanomaterials is leading to their inescapable release to the environment. Despite the scarcity of information related with their risks (Andreotti et al., 2015), mostly due to the difficulties involved in the quantification of NPs concentrations (Proulx and Wilkinson, 2014), an enormous concern regarding human health and environmental issues is rising (Markus et al., 2015).

Nanoparticles can enter in water systems directly through wastewater discharges, run-off and aerial deposition, and indirectly through atmospheric emissions and accidental spills during their manufacturing and transport (Fabrega et al., 2011; Baker et al., 2014). In fact, some metal oxide NPs were found in fresh waters namely, Fe, Zn, Mn and Ti nanoparticles (Pradhan et al., 2014).

The toxicity extension of CuO NP is still understudied but has been already reported that metallic NPs can cause oxidative stress, apoptosis and DNA-damage (Thit et al., 2015 and references therein) threatening the functioning of several ecosystems.

Estuaries are highly productive ecosystems (Gillanders et al., 2011; Jones et al., 2011) that have a crucial role in biogeochemical cycles (Jones et al., 2011) and unique physical-chemical conditions that support very dissimilar organisms and offer essential relations to near ecosystems (Sun et al., 2012). However, they are subjected to high anthropogenic pressures and they are considered sinks of contaminants (Ridgway and Shimmield, 2002; Spetter et al., 2015), which can affect their ecological functions. Thus, new tools and sustainable alternatives are in need to remediate and recover these coastal ecosystems.

Phytoremediation presents a solution to remove contaminants from natural media. This technique is based on the application of plants and associate microorganisms to remove, accumulate, metabolize, absorb and/or degrade organic and inorganic pollutants (Fulekar, 2012; Evangelou et al., 2015). Salt marsh plants and their associated microbial communities have an important role in the removal of organic and inorganic contaminants (Oliveira et al., 2014; Fernandes et al., 2015a, 2015b). In fact, these plants have potential to phytoremediate estuarine sediments contaminated with metals (e.g. Almeida et al., 2011), a process that can be affected if metals take the form of NP (Andreotti et al., 2015).

Moreover, microorganisms associated to plant roots can have a significant role on the phytoremediation potential of salt marsh plants. For instance, rhizobacteria of a natural wetland were able to promote Se and Hg bioaccumulation on wetlands plants (Chandra and Kumar, 2015), and bioaugmentation with microbial consortia enriched in metal resistant microorganisms promoted Cd phytostabilization by *Juncus maritimus* (Silva et al., 2014a) as well as Cd and Cu translocation in *Phragmites australis* (Oliveira et al., 2014; Silva et al., 2014a), two salt marsh plants commonly found in estuarine areas. Since NPs can be toxic to bacterial communities (Pradhan et al., 2014), evaluating if salt marsh rhizobacteria are affected by the presence of engineering NPs is necessary, because it can affect phytoremediation processes.

In this way, the aim of this work was to evaluate the response of the microbial communities colonizing *P. australis* and *Halimione portulacoides* rhizosphere to a contamination by CuO NPs and its implications for phytoremediation processes. We hypothesis that the metal form can condition the way the microbial communities in plants rhizosphere respond to the metal, which ultimately can have an effect on salt marsh plants phytoremediation potential for the remediation of Cu contaminated estuarine sediments. The effect of the metal form was assessed by comparing exposure to medium contaminated with Cu in ionic and in NP form. As a test medium, sediment from the rhizosphere of each salt marsh plants (rhizosediment) was soaked in the respective elutriate and contaminated with Cu in NP form or in ionic form, under controlled laboratorial conditions. With these sediment elutriates it is possible to estimate, throughout re-suspension processes, the amount of metal that is exchanged between sediment and the aqueous matrix.

The natural medium used has the advantage of simulating, in a simplified way, the interactions occurring in estuaries among water, sediment and organisms. Changes in terms of rhizosediment microbial community structure and abundance were determined. In parallel, plant's ability to uptake metal in both forms was also determined, using a similar medium, to evaluate the potential interactions between plants and NPs, as well as plants potential for future application in phytoremediation of NPs contaminated media.

H. portulacoides and *P. australis* were selected because they are frequently found in Portuguese salt marsh areas and they have ability to accumulate a high range of metals including Cu (Almeida et al., 2008).

2. Material and methods

2.1. Sampling

For the experiments, two sampling campaigns were performed in two different temperate estuaries in North of Portugal. The selected plant species do not co-habit the same salt marsh area in northern Portugal estuaries. In both estuaries a suitable amount of plants was collected so that only green plant specimens without a senescent appearance and with similar size and age were used.

The first campaign took place in May 2013 in Lima river estuary, a temperate salt marsh area, for the collection of *P. australis*, with its respective rhizosediment, and nearby estuarine water. Plants with sediment attached to their subterraneous structures (roots and rhizomes) (cubes of ca. $10 \times 10 \times 10$ cm^3) and water samples were transported to the laboratory, in decontaminated plastic bags and bottles, respectively. Later, in the laboratory, rhizosediment, sediment in contact with plants subterraneous structure, was removed by hand. Sediment not in contact with plants subterraneous structures was discarded. Rhizosediment was kept aside for the following experiments, described in Section 2.2. Small portions of this rhizosediment were separated. One portion was dried at room temperature for Cu determination (initial sediment) and another portion was directly store at −20 °C for posterior microbial community analysis. Plants roots and rhizomes were washed with deionized water and put in a nutrient solution (one quarter strength modified Hoagland nutrient solution (Hoagland and Arnon, 1950) until the beginning of the experiments (one day).

The second campaign took place in October 2013 in Cávado river estuary (temperate salt marsh area) for the collection of *H. portulacoides*.