Estuarine, Coastal and Shelf Science 141 (2014) 78–84

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Evaluation of the ability of two plants for the phytoremediation of Cd in salt marshes

Marta Nunes da Silva [a], Ana P. Mucha [b], A. Cristina Rocha [a], Carla Silva [a], Carolina Carli [a], Carlos R. Gomes [a], C. Marisa R. Almeida [b,*]

[a] CIMAR/CIIMAR, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal
[b] CIMAR/CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal

ARTICLE INFO

Article history:
Received 11 May 2013
Accepted 3 March 2014
Available online 12 March 2014

Keywords:
phytoremediation
salt marsh plants
Juncus maritimus
Phragmites australis
cadmium

ABSTRACT

Several salt marsh plant species have shown to be able to uptake and concentrate metals in their tissues, showing potential for metal phytoremediation. However, studies in controlled conditions, mimicking as much as possible the plants natural environment, are needed to confirm this potential. For the present study, *Juncus maritimus* and *Phragmites australis* were collected in an estuary together with the sediment surrounding their roots, put in vessels and maintained in greenhouses under estuarine tidal simulation. After 3 weeks of acclimation, vessels were spiked with two different cadmium concentrations. After 2 months, cadmium was assessed in plant tissues and sediments. Results indicate that both plant species were able to uptake and translocate cadmium into their tissues, contributing also to retain it in rhizosediments and thus reducing the available amount of metal in the environment. Metal was preferentially accumulated in belowground structures, in concentrations not directly proportional to the amount of cadmium present in the sediment. Although no visual toxicity signs were observed, some defence mechanisms were triggered as observed by the changes in carotenoids, lignin, total soluble phenolic compounds and thiolic compounds levels, this response differing between plant species. This work shows that these two salt marsh plants can contribute for the retention of cadmium in salt marshes being useful for the phytostabilization of this metal in estuarine environments.

1. Introduction

Salt marsh plants are known to immobilize and store metals in sediments surrounding their roots as well as in their below- and aboveground tissues (e.g. Fitzgerald et al., 2003; Jacob and Otte, 2003; Windham et al., 2003; Weis and Weis, 2004). In fact, several marsh plant species have been reported to concentrate metals in their belowground tissues at levels that exceeded those of the surrounding sediments (e.g. Almeida et al., 2004, 2006a; Cundy et al., 2005; Deng et al., 2006). This feature can be an advantage if these plants are used to clean and recover moderately impacted zones such as estuarine areas. In addition, these plants present efficient morphological and physiological mechanisms, which allow them to survive and reproduce in salt-rich environments (Manousaki and Kalogerakis, 2010). Salt marsh plants can therefore be suitable for phytoremediation.

Salt marshes in general have a relatively small number of highly productive plant species (Lefeuvre et al., 2003), including native and introduced ones. Introduced species can become problematic since some of them are considered invasive being therefore able of hampering the proper development of endemic species. Metal accumulation by salt marsh plants is highly dependent on the species and on the size and morphology of the plant root system (Doyle and Otte, 1997). For this reason, native and introduced plants can respond differently to the presence of metals in the environment. In fact, the presence of invasive and exotic plants in some areas of the salt marsh may considerably affect metal distribution and retention in the estuarine region, which will ultimately affect phytoremediation processes (Almeida et al., 2011).

Most studies on salt marsh plants ability to accumulate pollutants in their rhizosediments and tissues were carried out in the field and conclusions were drawn almost exclusively through the analysis of natural metal burdens in both salt marsh plant tissues

* Corresponding author.
E-mail addresses: calmeida@ciimar.up.pt, calmeida@fc.up.pt (C.M.R. Almeida).

http://dx.doi.org/10.1016/j.ecss.2014.03.004
0272-7714/

and sediment. However, different environments and different pollutant loads can significantly influence the obtained results. In addition, for a better understanding, experiments in controlled laboratory conditions have also been conducted. Nevertheless, such studies were carried out in non-natural media, like hydroponic systems (Reboredo, 1991, 2001), or in natural but simplified medium such as elutriate solutions (e.g. Almeida et al., 2008) or soil cultures. Therefore, studies that confirm salt marsh plants potential for metal remediation under controlled but environmentally relevant conditions are needed. In addition, these studies should take into consideration different plant species, including native and introduced ones, since these ecosystems present a natural high diversity in terms of flora.

The aim of this study was to evaluate the ability of two salt marsh plants, *Juncus maritimus* and *Phragmites australis*, for phytoremediating sediment contaminated with cadmium. This study was carried out in controlled conditions simulating as much as possible the natural estuarine environment. For that, plants were collected, put in vessels with their natural rhizosphere and maintained in a greenhouse with natural light and temperature conditions and with estuarine tidal simulation. The chosen plant species are halophyte and perennial, belonging to different taxonomic families and having, therefore, different morphological and physiological characteristics. *J. maritimus* is a sea rush from the family *Juncaceae*, being an autochthonous monocotyledon widely spread in salt marshes in the Atlantic coast of Europe. *P. australis* is a common reed from the family *Poaceae*. It is a rhizomatous perennial macrophyte found in wetlands throughout temperate and tropical regions of the world, which has invaded many coastal salt marshes, excluding most of other plant species (Havens et al., 1997). Studies in the field carried out by the authors with *J. maritimus* have shown enrichment factors for cadmium higher than 1 ($[Cd]_{root}/[Cd]_{sediment} > 1$), the value depending on the sediment characteristics (Almeida et al., 2004, 2011, 2006b). In addition, sediment in contact with the plant roots had in general a higher metal content than non-vegetated sediment. Similar results were also observed for *P. australis* ($[Cd]_{belowground\ tissues}/[Cd]_{sediment} > 1$) (Almeida et al., 2011). Such results indicate that both plants seem to contribute for the retention of this metal in the area of influence of their roots having, therefore, potential for phytostabilizing cadmium, a feature that deserves to be explored.

2. Experimental

2.1. Samples collection

J. maritimus and *P. australis* were collected at low tide in September 2011 in a site located in the Lima River estuary (NW Portugal).

Green plants, without a senescent appearance and with similar size and age, were collected together with the sediment surrounding their roots (cubes of approximately 20 cm × 20 cm × 20 cm) to preserve the plant rhizosphere environment. These plants were placed in plastic vessels adapted with plastic taps (6 vessels for each plant species). To minimize sediment losses through the taps, previously washed river pebbles were placed at the vessels bottom. Additional plants of each species were collected, also with sediment surrounding their roots, and stored in plastic bags for assessing field metal levels and plants' physiological parameters (see below).

Simultaneously, non-vegetated sediment, located within 0.5 m from the vegetated sediment, was collected. Cubes of sediment, of dimensions similar to the vegetated ones, were placed in similar individual plastic vessels (in a total of 6 vessels). A supplementary amount of non-vegetated sediment was also collected and stored in plastic bags for initial sediment characterization.

2.2. Microcosms experiments

Vessels with both plants and sediments were kept in two greenhouses, being exposed to natural light and environmental temperature conditions (greenhouses placed outdoors). To guarantee that the disposition of the greenhouses did not have any impact on the experimental conditions, the vessels were randomly arranged within and between greenhouses.

Vessels were watered through an automated irrigation system, regulated to mimic natural floods, being therefore under 2 daily tidal cycles, each one with two 6 h periods: one of flood and another of draught.

Irrigation solution consisted of a saline nutrient solution (adapted from Hoagland's nutrient solution).

After twenty days of acclimation (with tidal simulation), nine vessels (three per plant species plus three non-vegetated) were doped with a saline solution of 20 mg L^{-1} Cd^{2+} (as $CdCl_2$). The other nine vessels were doped with a saline solution containing 2 mg L^{-1} Cd^{2+}. These solutions were chosen to result in Cd concentrations in non-vegetated sediment and rhizosediments close to ERM and ERL levels, respectively (ERM = 9.6 μg g^{-1} and ERL = 1.2 μg g^{-1}, ERM being the sediment quality guideline that indicates the concentration above which adverse biological effects may frequently occur, and ERL the concentration below which adverse biological effects rarely occur (Long et al., 1995)). This Cd solution was kept in each vessel for 6 h, being afterwards collected into individual plastic bottles (one per vessel). In the next day the collected solutions were added to the respective vessel (to insure that all Cd in solution would be retained in the sediment) and drained out after 6 h. The vessels were kept in the greenhouses with tidal simulation, as above described, for 2 months after which they were dismantled.

2.3. Samples treatment

At the laboratory rhizosediment (sediment in contact with the plant belowground tissues) was carefully separated from the respective plant roots, being afterwards put to dry. Non-vegetated sediment was also dried at room temperature. Plants were divided into roots and rhizomes (belowground structures) and stems and leaves (aboveground tissues), which were carefully washed and put to dry at room temperature until constant weight. This procedure was followed not only for the dismantled vessels but also for the samples collected into bags at the sampling campaign.

In all vessels rhizosediments and plants tissues fresh and dry weight were recorded.

2.4. Analytical determinations

Cadmium content was determined in sediments, rhizosediments and in the different plants tissues. Samples were digested in a high-pressure microwave system (Ethos, Milestone) in Teflon vessels using concentrated HNO_3 (and 30% H_2O_2 solution, only for plants tissues) and analysed by atomic absorption spectrophotometry either with flame atomization (PU 9200X, Philips), or with electrothermal atomization provided with a Zeeman background correction (4100 ZL, Perkin–Elmer coupled to an ASTABLE 70 autosampler), depending on the metal levels, following procedures validated before in the laboratory (Almeida et al., 2004).

Total chlorophylls and carotenoids were extracted and quantified in *P. australis* leaves and *J. maritimus* stems, according to a modified protocol (Abadía et al., 1984). As *J. maritimus* leaves are very close to the sediment, stems were chosen for the analysis of