Available online at www.sciencedirect.com
SCIENCE DIRECT®
Estuarine, Coastal and Shelf Science 65 (2005) 191–198
ESTUARINE COASTAL AND SHELF SCIENCE
www.elsevier.com/locate/ECSS

Exudation of organic acids by a marsh plant and implications on trace metal availability in the rhizosphere of estuarine sediments

Ana P. Mucha [a], C. Marisa R. Almeida [a], Adriano A. Bordalo [a,b], M. Teresa S.D. Vasconcelos [a,c,*]

[a] *CIIMAR, Rua dos Bragas, 177, 4050-123 Porto, Portugal*
[b] *Laboratory of Hydrobiology, Institute of Biomedical Sciences, University of Porto, Largo Professor Abel Salazar, n° 2, 4099-003 Porto, Portugal*
[c] *Department of Chemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 687, 4169-007, Porto, Portugal*

Received 5 January 2005; accepted 10 June 2005
Available online 26 July 2005

Abstract

The aim of this work was to identify a variety of low molecular weight organic acids exuded by the sea rush *Juncus maritimus* collected at two locations with different sediment characteristics (sandy and muddy) and to examine whether specific differences in physico-chemical sediment characteristics influenced plant exudation. Just after collection, plant roots were rinsed and put in contact with deionised water for 2 h. In the obtained solution the organic acids, exuded by the plants, were determined by high performance liquid chromatography. *Juncus maritimus* was shown to be capable of releasing malonate and oxalate. Sediments and rhizosediments (sediment in contact with the plant roots and rhizomes, corresponding to the area of higher belowground biomass) from the areas where the plants had been collected were characterised in terms of physical and chemical composition, including acid volatile sulphide and total-recoverable metals (Pb, Cr, Cu, Zn, Ni and Cd). It was found that the extent of exudation varied markedly between sites. The identified organic acids were used as extractants of metals from sediments and rhizosediments and the results were compared with those provided by a very commonly used sequential extraction approach, which was carried out in parallel. This work demonstrates that *J. maritimus* can release organic compounds that can act as complexing agents of trace metal and therefore organic exudates should be accounted for when dealing with estuarine environment quality.

Keywords: root exudates; organic acids; trace metals; *Juncus maritimus*; estuarine sediments

1. Introduction

In urban estuaries, salt marshes often receive variable pollutant loads, including discharges of trace metals from industrial and transportation activities. When accumulated in marsh sediments, metals can be adsorbed and absorbed by plant roots and translocated to their above-ground biomass. Plant uptake of dissolved metals can reduce the input of metals into estuarine waters from sediments, and metal sequestration in plant tissues may provide a long term sink if metal-laden tissues are buried (Windham et al., 2003). The uptake of metals is affected by a number of environmental characteristics of sediments, namely the pH and redox potential (Burke et al., 2000).

The presence of vegetation and intense microbial activity at the root–sediment interface alters the concentration of O_2, CO_2 and organic matter (Otero and Macias, 2002), in such a way that those environments are

* Corresponding author. Department of Chemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 687, 4169-007, Porto, Portugal.
E-mail address: mtvascon@fc.up.pt (M.T.S.D. Vasconcelos).

0272-7714/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ecss.2005.06.007

considered as independent subsystems in the sediment, commonly referred as rhizosphere (Varennes, 2003). Differences in metal concentration and their chemical speciation in the vicinity of the roots of salt marsh plants have also been demonstrated (Caçador et al., 1996; Almeida et al., 2004).

The processes by which the plants modify metal speciation in the rhizosphere include mineral precipitation/dissolution (Sundby et al., 1998), changes in redox conditions (Madureira et al., 1997; Jacob and Otte, 2004a,b) and exudation of organic substances capable of complexing metals and changing their bioavailability (Parker et al., 2001; Berkelaar and Hale, 2003). This latter process is only well documented for plants with agricultural interest (Jones, 1998 and references therein). Organic acids, like malic, citric and oxalic acids, have been proposed to be involved in processes operating in the rhizosphere, including nutrient acquisition (Neumann et al., 1999), metal detoxification (Ma et al., 2001), alleviation of anaerobic stress in roots, mineral weathering and microbial attraction (Jones, 1998). Although it is assumed that salt marsh plants release exudates, knowledge about their nature, concentration and role on trace metals bioavailability in the rhizosphere is still scarce.

The aim of this work was to identify a variety of organic acids exuded by the sea rush *Juncus maritimus* and to examine whether specific differences in physico-chemical sediment characteristics influenced plant exudation. In addition, we studied the influence of the organic acids exuded by the plant on trace metal bioavailability in estuarine sediments, by using them as extractants, and the information provided was compared with that provided by a very commonly used sequential extraction (SE) procedure (Rauret et al., 1999), which was carried out in parallel.

2. Material and methods

2.1. Material and reagents

All reagents used were *pro analysis* grade or equivalent, except concentrated nitric acid, which was supra-pure. Filtered bi-deionised water (conductivity $<0.1\ \mu S\ cm^{-1}$) was used.

Standard solutions were prepared daily from the stocks, in polyethylene tubes, by weighing.

To prevent contamination, all materials used for sampling and sample treatments and all labwares were soaked in 20% (v/v) nitric acid solution for at least 24 h, rinsed several times with deionised water and dried in a Class 100 laminar flow hood. To collect the samples only plastic devices were used. The sample manipulation was carried out in a clean room with Class 100 filtered air.

2.2. Samples collection

Juncus maritimus is widely distributed in estuarine salt marshes of the NW Portuguese coast. Samples were collected in July 2003 during low tide, in the lower mesotidal Douro estuary (41 °N; 08 °W), at two sites with different sediment characteristics: one site located inside a small area of salt marsh, presenting high organic content muddy sediment, and the other located upstream the salt marsh, presenting low organic content sandy sediment. More detailed information on this estuary can be found elsewhere (Mucha et al., 2003a). Green plants without a senescent appearance and with similar size were collected at both sites, being carefully washed in seawater to remove debris and dead root and shoot material. Concomitantly, sediment and rhizosediment (sediment in contact with the plant roots and rhizomes, corresponding to the area of higher belowground biomass) were collected between 10 and 20 cm depth, which correspond to the roots depth. Each sample was individually packed in a plastic bag by using plastic shovels (rhizosediment and sediment) or plastic gloves (for plants) and carried to the laboratory within 30 min.

At the laboratory, plant roots were rinsed thoroughly with deionised water and then rinsed in deionised water containing the antimicrobial agent Micropur®, to stop microbial degradation of the exudates during the collection. In preliminary tests, two antimicrobial agents, Micropur® and sodium hypochloride, were used in parallel, as well as rinsing only with deionised water. No significant differences were observed in the results obtained for plants washed with Micropur® and with only water, but sodium hypochloride significantly increased exudation, and was considered aggressive to plant roots. Despite the absence of differences for plants washed with deionised water and with Micropur®, we decided to use the latter in order to assure the absence of microbial degradation.

2.3. Organic acids exudation

Six groups (3 replicates per site), each with 10 freshly collected plants, were prepared and the respective roots were immersed in 100 ml of deionised water for 2 h. This method has been recently used for collection of root exudates (e.g., Fan et al., 1997) from plants grown in nutrient solution, and allows the identification of possible exudates, but not the quantifications of exudation rates due to possible osmotic effects on the roots. The dry weight of the roots per group was determined as being 0.79 ± 0.20 g. The length of the exposure time was selected from a set of previous experiments with exposure times of 1, 2, 3 and 24 h. No significant differences were observed in the solution composition obtained after 1, 2 and 3 h of exposure, whereas for the 24 h experiment a marked decrease in the volume of solution was