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Multi-element accumulation near *Rumex crispus* roots under wetland and dryland conditions

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Patterns of element accumulation near the roots of plants differ between dryland and wetland conditions.

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ABSTRACT

Rumex crispus was grown under wet and dry conditions in two-chamber columns such that the roots were confined to one chamber by a 21 μm nylon mesh, thus creating a soil–root interface ('rhizoplane'). Element concentrations at 3 mm intervals below the 'rhizoplane' were measured. The hypothesis was that metals accumulate near plant roots more under wetland than dryland conditions. Patterns in element distribution were different between the treatments. Under dryland conditions Al, Ba, Cu, Cr, Fe, K, La, Mg, Na, Sr, V, Y and Zn accumulated in soil closest to the roots, above the 'rhizoplane' only. Under wetland conditions Al, Fe, Cr, K, V and Zn accumulated above as well as 3 mm below the 'rhizoplane' whereas La, Sr and Y accumulated 3 mm below the 'rhizoplane' only. Plants on average produced 1.5 times more biomass and element uptake was 2.5 times greater under wetland compared to dryland conditions.

1. Introduction

In contrast to 'dryland' plants, many wetland plants display constitutive tolerance to elevated metal concentrations in the soil, meaning that they are tolerant to metals regardless of the metal concentrations at their location of origin (Matthews et al., 2004; McCabe et al., 2001; McNaughton et al., 1974; Ye et al., 1997a). Otte and co-workers (McCabe et al., 2001; Otte et al., 2004) suggested that the development of metal tolerance in wetland plants may be attributed to the biogeochemistry of wetland substrates. They proposed that the formation of Fe plaque deposits in the vicinity of wetland plant roots contributes to higher metal mobility and thus greater metal accumulation near plant roots. As a consequence wetland plants have been exposed to higher concentrations than dryland plants over the course of evolution, which favored selection for constitutive metal tolerance.

The bioavailability and mobility of chemical elements are influenced by changes in soil properties surrounding living plant roots including pH, organic content, cation exchange capacity, redox potential (Eh), moisture status and temperature (Alloway, 1995; Davies, 1994; Jacob and Otte, 2003). Plant roots influence the environment directly adjacent to them in order to obtain access to nutrients, in particular the essential macro- and micro-nutrients (Inderjit and Weston, 2003; Jungk, 2002; Marschner et al., 1986; Mehra and Farago, 1994; Neumann and Romheld, 2002). Wetland plants can modify redox conditions, pH and organic matter of the soil or sediment and thus affect the mobility (Wright and Otte, 1999) and chemical speciation of metals in waterlogged environments (Jacob and Otte, 2003). Knowledge of the biogeochemistry of metals and the processes affecting their mobility and trophic transfer is important, (1) because of their potential ecotoxicological effects, (2) because recent research has shown that less-studied elements such as the rare earth elements may be beneficial to plant growth (Chang, 1991; Hong, 2002), and (3) because of the increasing demand for less-studied metals, such as the rare earth elements, for development of new technologies and their subsequent potential environmental impacts.

Hinsinger and Courchesne (2008) emphasize that rhizosphere studies play a key role in research on the biogeochemistry of elements. Most rhizosphere studies have used dryland plants such as *Brassica napus* (rape) (Kuchenbuch and Jungk, 1982), *Hordeum vulgare* L. var. Dorirumugi (barley) (Youssef and Chino, 1989b, 1991) and *Glycine max* (soybean) (Youssef and Chino, 1991). The few studies using wetland plants include *Oryza sativa* L. (rice) (Begg et al., 1994; Kirk and Bajita, 1995), *Halimione portulacoides* (sea purslane), *Spartina townsendii* (cordgrass) (Doyle and Otte, 1997), *Spartina anglica* (common cordgrass) (Otte et al., 1995) and *Typha latifolia* (narrow leaf cattail) (Jacob and Otte, 2004). But none of these studies have compared element concentrations across the rhizosphere under

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flooded (wetland) and non-flooded (dryland) conditions. The aim of this study was to investigate the hypothesis that metals accumulate in the direction of plant roots in flooded soil more than in non-flooded soil and that this would lead to greater uptake of those metals in plants.

2. Materials and methods

2.1. Seed collection and soil preparation

Mature *Rumex crispus* fruits were collected in West Fargo, North Dakota (N 46° 52′ 30.7″, W 96° 58′ 08.7″) in October, 2006, and stored at 5 °C (Baskin and Baskin, 1978) for 5 months. The seeds were washed in distilled water after sepal removal and germinated on moistened sterile sand for 2 weeks in an incubator (14 h photoperiod, 25 °C). The seedlings were planted in 5 cm potting soil (Sun Gro Sunshine LG3 germinating mix with vermiculite) and allowed to develop roots for about 6 weeks in a greenhouse (16 h photoperiod, 3.03 log lumen m^{-2} (mean day time), 20–30 °C).

Local farmland soil was obtained from near Casselton in Cass County, North Dakota (N 46° 50′ 51.4″, W 97° 09′ 20.0″, 280 m). This soil was selected because it was more representative of natural conditions compared to substrates such as potting soil or sand. The soil was determined to be a silty clay with 4.1% organic matter, bulk density of 1.04 g cm^{-3} and particle size 5.8% Sand, 47.9% Silt and 46.3% Clay (North Dakota State University Soil Testing Lab). The soil was oven-dried (60 °C) to constant weight, crushed and passed through a 2 mm screen. The soil was amended with sterile sand (Quikrete Premium Play Sand) at a ratio of 3:1 soil to sand (by weight) to aid root penetration in the clay-rich soil.

2.2. Column apparatus assembly

The columns consisted of 2 sections of soil which were separated by 21 μm nylon mesh (Nylon 21/17, Miami Aqua-culture, Inc.). The mesh restricted root growth to the upper section of the column while allowing diffusion of nutrients and water throughout the soil. The mesh was considered the rhizoplane because it separated the roots from the soil in the lower section of the column. This design enabled soil sampling in two different regions of the soil column; 1) above the rhizoplane (upper section of column) and 2) below the rhizoplane at distinct distances (lower section of column) (Fig. 1).

The columns consisted of PVC pipe (9 cm diameter) cut into two sections measuring 10 cm (lower section) and 6 cm (upper section). The mesh was attached to one end of the 6 cm section. The two-chamber column was assembled by securing the 10 cm section to the 6 cm section with 5-cm wide waterproof Duct Tape (Nashua® Tape) with the nylon mesh in between. Both sections were filled with the prepared, homogenized soil/sand mixture, the lower section with 600 g to which 300 ml of distilled water was added evenly and the upper section with 300 g to which 150 ml of distilled water was also added evenly. To prevent soil loss from the column and still allow water movement, a 2 mm mesh was secured to the bottom end. The lower section of the column was inverted, filled with soil and secured with the 2 mm mesh before soil was added to the upper section to ensure contact between the bottom soil and the rhizoplane. The seedlings were removed from the potting soil, washed gently with distilled water and planted in the upper section of the column (1 seedling per column).

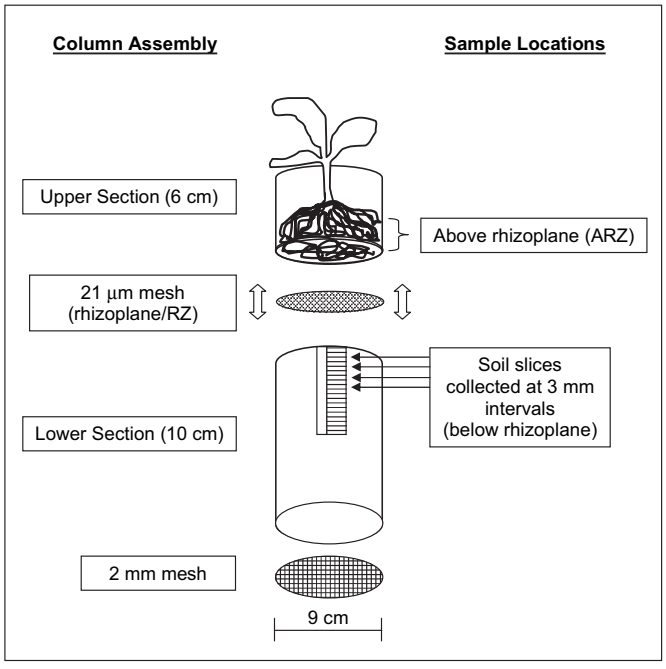

Fig. 1. Column apparatus for *Rumex crispus* experiment showing column components on the left and sampling locations on the right.

2.3. Soil flooding and monitoring moisture

This experiment was carried out in a greenhouse and the treatments arranged using complete randomized design. The columns were placed into 2 L containers. The plants were allowed to establish for two weeks prior to beginning the moisture treatments. The flooded treatment ($n = 10$) consisted of adding distilled water to the containers such that the surface of the soil was below 5 mm of water. The non-flooded treatment consisted of columns ($n = 10$) that received water as needed according to their wilting point weights (see below).

Both treatments were monitored daily to determine when water addition was necessary. Sterilized cotton wicks with one end in sealed bottles of distilled water and the other end inserted into the soil above the rhizoplane were used continuously to maintain saturation of the flooded treatment. The same approach was used for the non-flooded treatment when water addition was necessary. The wicks were inserted in the soil in the upper section of the column and spread between the rhizoplane and the soil above the rhizoplane with any exposed portion of the wick wrapped securely with plastic. Water levels for the flooded treatment were restored when necessary to the marked lines of the initial water level (water was added to the larger container outside the column). The weights and plant height of the non-flooded treatments were monitored daily to determine if they were within 1 g of the wilting point weight. The wilting point had been determined previously by saturating the soil of four *R. crispus* plants growing in columns and then allowing the soil to dry. The weights of the columns containing plant and soil when the plant showed signs of wilting were determined. These weights were used to calculate an estimate of the weight of a column containing wilted plant and soil. *R. crispus* plants grown for 8 weeks were assessed for their height and weight which was used to obtain a linear equation with which to make adjustments when calculating the soil weight in the columns.

2.4. Soil sampling – pH and redox potential measurements

After 13 weeks, soil samples were collected from columns selected in random order. Each column was cut carefully to separate the upper and lower sections. The plant and soil in the upper section of the column were removed and soil was shaken from the roots. The soil remaining on the roots was collected and considered 'above rhizoplane' soil. The soil immediately below the nylon mesh (rhizoplane) was sampled using 60 ml syringes with the tips removed so they became small soil corers (2.5 cm diameter). The column was inverted and 3 syringes were inserted into the soil at the center, away from the column edges. The soil was extruded from each syringe in 3 mm intervals, sliced carefully and retained for analysis. Seven samples were collected from each syringe at 3, 6, 9, 12, 15, 18 and 21 mm intervals. Samples from the three syringes were pooled for each increment to obtain enough soil (at least 3 g) for analysis.

Immediately upon obtaining a sample, pH and Eh were measured using a VWR Symphony SP90M5 Handheld Multimeter. Approximately 1 g of fresh soil sample was used to determine the soil pH in a 1:2 soil:water ratio (Gavlak et al., 2003). A soil paste (about 500 mg fresh soil sample and 3 ml water) was used to measure the Eh (Patrick et al., 1996).

2.5. Ferrous iron (Fe^{2+}) concentration and multi-element analysis of soil and plants

Fe^{2+} concentration was determined using a method modified from Roden and Wetzel (1996). Fe^{2+} standards were prepared from a stock solution containing 100 mg L^{-1} $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ in 1% (v/v) 6 M HCl. A fresh soil sample of known weight (about 0.5 g), was immediately transferred to 5 ml of 0.5 M HCl and extraction allowed overnight. The extraction was then filtered (0.45 μm pressure filter, Pall Corporation Supor®-450), diluted (flooded samples – 1:40 dilution, non-flooded samples – 1:10 dilution) and 0.25 ml of the diluted sample or of standard was added to 1.25 ml of FerroZine solution (1% wt/wt FerroZine in 50 mM HEPES buffer). After about 5 min, the absorbance was measured using a Helios Gamma UV–Vis Spectrophotometer at $\lambda = 562$.

The remaining soil was oven-dried (60 °C) until constant weight, crushed using mortar and pestle and homogenized. The samples were then analyzed for multiple elements (37 elements) via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) by a commercial laboratory (Activation Laboratories, Ltd, Analysis by Aqua Regia Extraction with ICP/OES finish). Method detection limits in mg kg^{-1} were as follows; Ag 0.2; Al 100; As 2; B 10; Ba 10; Be 0.5; Bi 2; Ca 100; Cd 0.5; Co 1; Cr 1; Cu 1; Fe 100; Ga 10; Hg 1; K 100; La 10; Mg 100; Mn 5; Mo 1; Na 10; Ni 6; P 10; Pb 7; S 100; Sb 2; Sc 1; Sr 1; Te 1; Ti 100; Tl 2; U 10; V 1; W 10; Y 1; Zn 2 and Zr 1 (Accredited Laboratory; ISO/IEC 17025:2005).

The plants were washed gently in distilled water, separated into aboveground and belowground material, oven-dried (60 °C) until constant weight, crushed and