

Spatial variability of cadmium absorption in intact roots of sunflower (*Helianthus annuus* L.)

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- Agricultural soils are increasingly contaminated by Metallic Trace Elements (MTE), mainly depending on anthropogenic activities. Crops take up MTE from the soil solution and these contaminants are then transferred to edible plant parts. In 2009, the European Food Safety Authority (EFSA) recommended to divide by three the human exposure to Cadmium (Cd) from food. Subsequent decreases in the regulatory thresholds for Cd in crop products are expected, in particular for sunflower, which generally accumulates more Cd than other common crops such as maize. This may be a serious concern for farmers, who may encounter difficulties to grow sunflower, the grains of which satisfy the regulatory thresholds. Therefore, understanding how the root system takes up Cd is important to manage crop contamination. In the literature, some studies reported, in different species, a longitudinal variability in the root absorption capacity for different elements, including Cd, the absorption decreasing significantly with the distance from the root tip. Hence, a highly branched root system is expected to absorb more Cd. This work aimed at investigating if such a longitudinal variability in Cd uptake also exists in sunflower.
- Because of the difficulties to study Cd absorption in intact roots grown in soil conditions, experiments were conducted in hydroponics. The root system of intact 10-12 leaves sunflower plants were exposed for 20 minutes to a ¹⁰⁹Cd-labelled solution having individual Cd concentrations ranging from 0.7 to 105 nM. After removal of the Cd sorbed onto the root surface, individual first order laterals emerging from the taproot were subsampled and cut into segments to map the absorbed ¹⁰⁹Cd. A preliminary experiment demonstrated that there was no significant longitudinal transfer of the absorbed ¹⁰⁹Cd during the 20 minutes of exposure.
- Whatever the root segment, the Cd influx (nmoles/cm²/s) was a linear relationship of the Cd concentration in the solution, indicating that the absorption capacity was not saturated. The slope of the relationship, which accounts for the capacity of the root segment to absorb Cd, differed significantly between root segments, showing a decrease in the capacity of Cd absorption with the distance from the root tip: the Cd influx of the roots tips was about three times higher than that of basal segments.
- The absorption of Cd by the second order laterals was mathematically simulated by assigning to them the longitudinal profile of the absorption properties of the first order laterals. The simulated uptake fitted well the experimental values, suggesting that the first and second order laterals have similar profile of Cd absorption capacities along the root axis.
- The decline in the capacity of root tissue to absorb Cd with the distance from the root tip might have several origins. Photoassimilates are generally more concentrated around the root apex, providing a greater availability of energy for the active uptake of Cd by root cells. On the other hand, apoplastic barriers (Caspary bands, and suberin lamellae), which limit Cd transport in the apoplast, have a basipetal maturation. Hence, by being more mature at the basal part of the root axis, they might limit the radial transport of Cd in the apoplast and consequently the internalisation of Cd by the inner root cells. It has been shown that the development of apoplastic barriers are strongly depending on the root environment, in particular, soil conditions are expected to have a significant effect on the development of these barriers as compared to hydroponics. Therefore, the relationship between the root profile of Cd absorption and the development of apoplastic barriers is now being studied to assess if the longitudinal variability in Cd absorption by individual roots could significantly impact the Cd uptake by the whole root system of sunflower depending on its architecture.

Key words: cadmium - root absorption - spatial variability - sunflower.

INTRODUCTION

Agricultural soils are increasingly contaminated by Metallic Trace Elements (MTE) (Marseille, 2000) mainly depending on the spreading of pesticides, organic or mineral fertilizers and amendments (Adriano, 2001). Crops take up MTE from the soil solution and these contaminants are then transferred to edible plant parts. For food safety purpose, the European Food Safety Authority (EFSA) revised in January 2009, the previous recommendation for the diet exposure of human to Cd. The revised value is about three times as low as the previous one that was established by the World Health Organisation (WHO): from 7 to $2.5\mu\text{g}\cdot\text{kg}^{-1}$ body weight $\cdot\text{week}^{-1}$. Subsequent decrease in the regulatory thresholds for Cd crop products is expected, in particular for sunflower, which generally accumulates more Cd than other common crops such as maize (Broadley et al, 2001). This may be a serious concern for farmers, who may encounter difficulties to grow sunflower the grain of which satisfy the regulatory thresholds. Therefore, indicators of Cd absorption by plants are necessary to manage crop contamination.

Many indicators have already tested, such as total or mobile MTE concentrations in soils, extracted by chemical reagents like neutral salts, EDTA, and acids (Gupta and Aten, 1993). However, studies have demonstrated their failure to be robust predictors of crop contamination (Nolan et al, 2005; Tambasco et al, 2000). Consequently, a mechanistic modelling of the soil to crop transfer of MTE is necessary, including a better understanding of how root systems take up Cd.

In the literature, some studies reported in different species a longitudinal variability in the root absorption capacity for NH_4^+ (Colmer and Bloom, 1998), phosphorus (Ferguson and Clarkson, 1976), and Cd^{2+} (Pineros et al, 1998), the absorption decreasing significantly with the distance from the root tip. Hence, a highly branched root system is expected to absorb more Cd. No data are available for sunflower (*Helianthus annuus L.*). Therefore, this work aimed at investigating if such a longitudinal variability in Cd uptake also exists in roots of sunflower.

In the present work, we used ^{109}Cd labelling experiments with Cd concentrations within the range of that found in agricultural soils (Sauve et al, 2000) in order to study the Cd root uptake properties of first and second order lateral roots.

MATERIALS AND METHODES

Plant culture

Because of the difficulties to study Cd absorption in intact roots grown in soil conditions, experiments were conducted in hydroponics. Sunflower (*Helianthus annuus L.*) cv ES Biba was grown in hydroponics in $\frac{1}{4}$ strength Arnon & Hoagland nutrient solution in greenhouse. All experiments used 10-12 leaves plants.

Preliminary experiment: significance of the longitudinal transfer of ^{109}Cd root during the exposure

A preliminary experiment was conducted to check that the radioactivity found in a root segment corresponded to the total absorbed Cd i.e that there was no significant longitudinal transfer of the absorbed ^{109}Cd during the labelling duration. A single root still attached to the plant was isolated and five drops (40 μl each) of a 500 nM Cd buffered solution were deposited at 0.5 cm interval, for 5 min, 10 min, or 20 min. One of the 5 drops located either at the root apex (the first drop) or at a more basal segment before branching (the third drop), was labelled with ^{109}Cd . Other unlabelled drops were deposited as neighbours of the labelled drop to investigate a possible transfer. After the exposure, the five drops of solution were removed and the corresponding root segments were sampled and placed individually in 2 successive ice-cold desorption baths (for 5 min each) containing 0.05 mM of unlabelled Cd and 5 mM of calcium, in order to remove the Cd sorbed onto the roots. Each segment was mineralised by wet digestion in a 65% nitric acid and 70% perchloric acid) mixture (1/1) at 60°C, and its radioactivity was determined by liquid scintillation counting.

Mapping of Cd influx in first order laterals

The whole root system of an 10-12 leaves intact sunflower plant was first equilibrated for 5 min in two successive 2 L solutions with Cd concentrations ranging from 0.7 to 105 nM (n=5). The root system was then transferred for 20 minutes in a third bath having in addition carrier-free ^{109}Cd . Afterwards, in order to remove the Cd sorbed onto the roots, the root system was deeped into two successive ice-cold desorption baths (the same as in the preliminary experiment). Then one representative first ordered lateral was detached from the tap root and sliced into successive axial segments. Its laterals were individually sampled. All root parts were scanned to determine their length and average diameter. Root segments were individually digested by wet digestion (see preliminary experiment) to determine their radioactivity. After

having determined their individual length and average diameter from scanning, the second order laterals were pooled and mineralised to determine the Cd they absorbed.

Modelling of Cd absorption by second order laterals

The uptake of Cd by second order laterals was simulated by applying to them the longitudinal profile of Cd influx determined in the first order laterals and by using their individual morphological characteristics (diameter and length). Simulated uptakes were compared to values measured experimentally.

RESULTS AND DISCUSSION

Longitudinal transfer of Cd during the exposure time is not significant

A significant radioactivity was found in the root segments adjacent to the labelled one but the amount found was less than 1% of the total recovered. This showed that for up to 20 min of exposure, Cd was little mobile in roots and that, in our experimental conditions, longitudinal transfers of Cd could be ignored in our work (Figure 1).

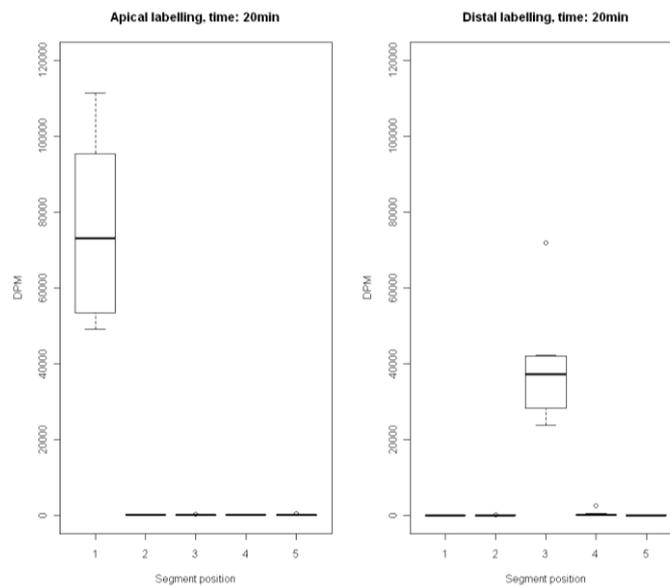


Figure 1. ¹⁰⁹Cd radioactivity (DPM=disintegration per minute) recovered in the different root segments.

Spatial variability in Cd influx in first order lateral

Whatever the root segment position, the root influx of Cd ($\text{nmol}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$) was a linear relationship of the Cd concentration in the solution. This result indicated that the absorption capacity was not saturated for the low Cd concentrations tested here, so the theoretical Michaelis kinetics could be approximated by a linear model (Figure 2).

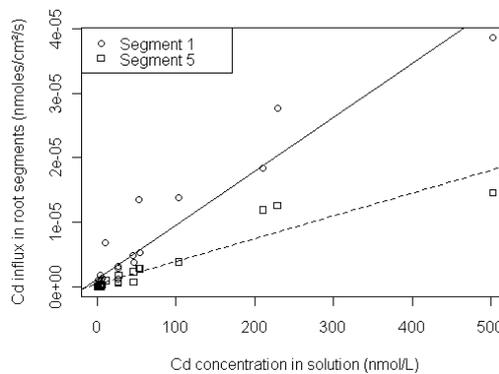


Figure 2. Influx of root segments 2 and 5, as a function of the Cd concentration in the solution.

The response of the influx to the Cd concentration in the solution (the slope of the linear model=the specific absorption of Cd) depended on the position of the root segment (Figure 3): the specific absorption of Cd was about three times higher for apical segments than for basal segments. These values were consistent with those found in the literature for other non hyperaccumulator species. For example, in maize the specific absorption of Cd for our concentrations was about $2.2 \cdot 10^{-7} \text{ L}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ (Redjala, 2009). In Figure 3, the specific absorption parameter was the slope of the relationship between the influx of Cd and the Cd concentration in the solution (see Figure 2).



Figure 3. Evolution of the specific absorption of Cd as a function of distance to root apex in sunflower.

The decline in the capacity of root tissue to absorb Cd with the distance to the root tip might have several origins. Photoassimilates are generally more concentrated around the root apex, providing a greater availability of energy for the active uptake of Cd by root cells. On the other hand, apoplasmic barriers (Caspary bands, and suberin lamellae), are known to limit the diffusion of elements within the apoplasm such as phosphorus (Ferguson and Clarkson, 1976). The apoplasmic barriers have a basipetal maturation. Hence, by being more mature around the basal part of the root axis, they might limit the radial transport of Cd in the apoplast and consequently the internalisation of Cd by the inner root cells.

Root uptake properties of second order laterals

The simulated versus observed influx of Cd in second order laterals was a linear relationship having a slope not significantly different from 1:1 ($R^2=0.92$) (Figure 4). In Figure 4, the 2nd order laterals Cd absorption was simulated by using the longitudinal profile of the absorption properties found in the first order laterals. This suggested that the absorption properties of the second order lateral were similar to that of the first order laterals.

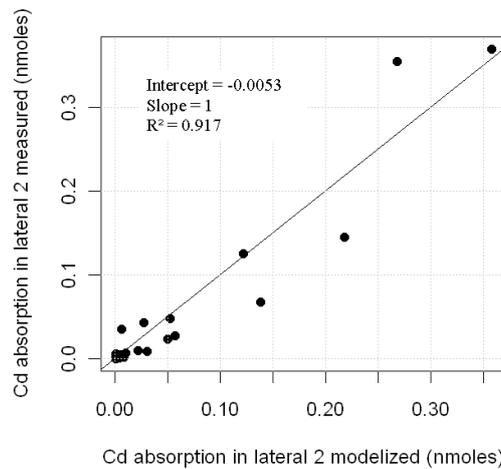


Figure 4. Observed versus simulated absorption of Cd by 2nd order laterals of sunflower.

This work showed that the Cd influx of sunflower roots was variable along the root axis with a decrease by 3 times between apical and basal parts of the root. Furthermore, we found no evidence that absorption properties were significantly different between the first and the second order lateral roots. It has been shown that the development of apoplasmic barriers are strongly depending on the root environment, in particular, soil conditions are expected to have a significant effect on the development of these barriers as compared to hydroponics (Redjala et al, 2011). Therefore, the relationship between the root profile of Cd absorption and the development of apoplasmic barriers is now being studied to assess if the longitudinal variability in Cd absorption by individual roots could significantly impact the Cd uptake by the whole root system of sunflower depending on its architecture.

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