Is the hyperaccumulating plant Arabidopsis halleri a good candidate for phytoextraction ?

Stéphanie Huguet, Géraldine Sarret, Agnès Laboudigue, Véronique Barthes, Valérie Bert

Is the hyperaccumulating plant Arabidopsis halleri a good candidate for phytoextraction?

S. Huguet[1,2,3,5], G. Sarret[2], A. Laboudigue[1,3], V. Barthès[4], V. Bert[1,5]

[1] CNRSSP Centre National de Recherche sur les Sites et Sols Pollués, 930 Bd Lahure, BP 537, 59505 DOUAI Cedex France, huguet@cnrssp.org

[2] LGIT, Geochimie de l'Environnement, Université de Grenoble et CNRS, BP 53, 38041 Grenoble Cedex 9, France

[3] Département Génie Civil et Environnemental, Ecole des Mines de Douai, 941 Rue Charles Bourseul, 59508 Douai Cedex, France

[4] CEA/LITEN/DTNM/L2T, CEA Grenoble, Av des Martyrs, 38054 Grenoble cedex 9, France

[5] INERIS Unit Déchets et Sites Pollués, Parc Technologique Alata, BP2, 60550 Verneuil-en-Halatte, France

Key words: *Arabidopsis halleri*, cadmium (Cd), hyperaccumulation, phytoextraction, speciation, sediment

Abstract

The cleaning of waterways by regular dredging generates great volumes of sediments and, owing to human activities, these sediments often contain large amounts of metals (Cd, Zn, As, Pb) which are potentially toxic for plants and animals. Phytoextraction could be a solution to treat this kind of sediments. This work focuses on the mechanisms of Cd accumulation in *Arabidopsis halleri*, a Cd and Zn hyperaccumulator, and the effects of this species on a metal polluted sediment.

Introduction

In the north part of France, the maintenance of waterways by regular dredging operations generates large volumes of sediments that often contain large amounts of metals (Cd, Zn). The absence of effective and low cost technologies to treat polluted sediments creates the need to develop innovative technologies for remediation. Since the last ten years, many studies were dedicated to the potential use of plants in order to extract, contain or immobilize metals in contaminated soil. Some plants called hyperaccumulators are able to accumulate high amounts of metals in their aboveground parts. This interesting plant property could be used for phytoextraction. The lack of knowledge of the mechanisms involved in metal hyperaccumulation is a major restraint for phytoextraction development.

This project is focused on Cd accumulation mechanisms in the Zn and Cd hyperaccumulator *Arabidopsis halleri* and combines physics, physicochemical and biological approaches.

Materials and methods

Sediment: Dredged sediment was collected from the Scarpe canal in the Nord Pas de Calais Region. This area is heavily polluted with Cd (around 140 mg · kg^{-1}). Sediment was air dried and mechanically homogenized to reduce its water content. Pots (4l) were filled with sediment and equipped to collect leachates (Photo 1).

Plant material: *A. halleri* developed natural populations on both metal contaminated and uncontaminated soils and sediments in France and Belgium (Bert *et al.*, 2002). Seeds of A. halleri from a metallurgical waste (pH = 6.8 - Auby, Fr) and from an uncontaminated soil (Hautes Fagnes, Be) were germinated on compost for 4 weeks. Seedlings were transferred to the sediment pots after roots have been washed carefully with deionized water (Photo 1). Seedlings of white mustard (*Brassica napus*) were grown in the same conditions as a non-accumulator control. Seedlings were also transferred to pots filled with agricultural soil to have a plant growth control. The design of the experiment is one seedling per pot and three replicates per origin and plant species. Soil and sediment moisture was maintained at 80% field water capacity with additions of regular de-ionised water after weighing. Plants were grown for 6 months in a controlled environment growth room (12h photo period; 20/16℃ day/night temperature; 80% relative humidity).

Figure 1. *Arabidopsis halleri* "Auby" growing on contaminated sediment in the growth room and non vegetated pots (sediment control).

Pot experiment: Before the start of the experiment, total metal concentrations in the sediment were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after microwave HCl/NO_3 digestion. Once a month, leachates were collected, pH was measured, and their composition was analysed. Cadmium phytoavailability was performed once a month using DGT probes (Nolan *et al.*, 2005) and $Ca(No_3)_2$ extraction. Health status and plant growth were regularly monitored by counting new leaves and measuring the size of the rosette. Total Cd concentration in leaves was measured once a month by ICP-AES.

Hydroponic culture

Cuttings from a plant from Auby and from a plant from Hautes Fagnes were grown in hydroponics with the following basal nutrient solution (0.5 mM $MgSO_4 \cdot 7H_2O$, 1 mM $NH_4H_2PO_4$, 2 mM $Ca(NO_3)_2 \cdot 4H_2O$, 3mM KNO_3, 0.1μM $(NH4)_6Mo_7O_{24}$, H_2O, 0.1μM $CuSO_4 \cdot 5H_2O$, 25μM H_3BO_3, 2μM $MnSO_4 \cdot H_2O$, 1μM KCl, 0.1μM NaCl, 30μM $ZnSO_4 \cdot 7H_2O$ and 20μM Fe(III)-EDDHA). White mustard was also cultivated in hydroponics as a non-accumulator plant control. Clones were used in two experiments: one aims at quantifying Cd accumulation in leaves, the other one aims at localizing Cd distribution at the plant scale by autoradiography. Cadmium was added as $CdCl_2 \cdot 2.5H_2O$ in the nutrient solution after 4 weeks of growth in hydroponics.

Cd Autoradiography: *A. halleri* (Auby) plants previously studied in Sarret *et al.* (2002) were grown in hydroponics added with 20μCi/L ^{109}Cd and various concentrations of non-radioactive Cd (0, 10 and 50μM), non-radioactive Zn (0, 30μM and 2 mM) for various times (2, 3 and 5 weeks). Each experiment was done in triplicate.

Another experiment was designed and is in progress. In the nutrient solution, 20μCi/L ^{109}Cd was added in each pot. Cadmium (0, 5 and 20μM) and/or Zn (30, 300μM) were added in the pots depending on the aim of the experiment. Each experiment was done in triplicate too, with three plants growing in the same pot. After various times of exposition (1, 2, 3 and 9 weeks), aboveground parts of plants were cut and arranged as flat as possible on a paper film and covered with a polyethylene film. To obtain autoradiographs of adaxial side, SR autoradiography films (Packard) were placed against the polyethylene film. After overnight exposition, films were developed using a Cyclone Storage Phosphor System, and obtained autoradiographs were treated with Optiquant and ImageJ softwares.

Results

Pots experiment: The experiment is still in progress. The aim of the experiment is to quantify the impact of the growth of A. halleri from two origins (metallicolous and non metallicolous) on a metal contaminated sediment in terms of Cd accumulation in leaves and Cd speciation in the sediment.

Cd distribution in leaves of Arabidopsis halleri (Auby, preliminary results)

The distribution of Cd and Zn was firstly studied at the scale of the whole plant at various exposure times. After 2 weeks of exposure to Cd only, Cd was accumulated mostly in new leaves and veins of oldest leaves. After 5 weeks, Cd was still distributed in new leaves, but also in all tissues of the oldest leaves.

When plants were exposed to Cd and Zn, a more homogeneous distribution of Cd among new and oldest leaves was observed.

The distribution of metal was then investigated at the leaf level. After 3 weeks of exposure, trichomes of *A. halleri* were enriched in Cd (Figure 2) and showed a four-fold higher activity than the leaf tissue ($1.68.10^6$ Digit Light Units (DLU) per mm^2 and $4.44.10^5$ DLU/mm^2, respectively). Trichomes represent 2.5 % of the total leaf area, which corresponds to 8% of Cd, the rest being sequestered in the leaf tissue. Therefore, in *A. halleri* trichomes represent a minor compartment of Cd accumulation, which is consistent with previous observations (Sarret *et al.*, 2002).

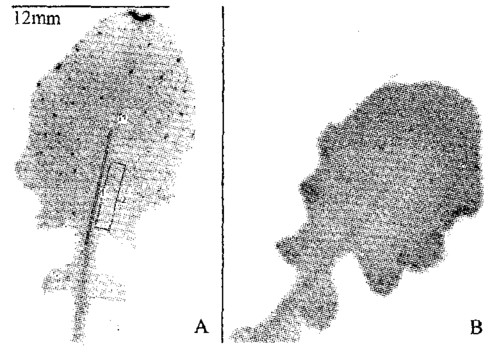

Figure 2. Autoradiographs of leaves of *A. halleri* exposed to 10μM Cd, 2mM Zn and 20μCi/L ^{109}Cd for 3 weeks (a) and for 5 weeks (b). Black spots are trichomes.

Discussion

Autoradiography was used in very few studies on metal accumulation in plants. Preliminary rests showed that autoradiography was an interesting tool to visualize Cd distribution and accumulation zones in the leaves with a good lateral resolution, and quantify Cd activity in single leaves. However, this technique did not allow a precise quantification of total Cd content in the plant. This latter parameter will be measured by ICP-AES in a parallel experiment with stable Cd and Zn.

Zhao *et al* (2006) observed that Zn inhibited short-term uptake of Cd in metallicolous *A. halleri*. This result will be compared with our results obtained by autoradiography. The effect of an increase in Cd concentration will be investigated too. These different phenomena will be observed on a longer time (9 weeks), and compared with the non-metallicolous *A. halleri* species. Variation in Cd accumulation among different populations may be expected (Bert *et al.*, 2002).

Acknowledgements

This work was supported by the Nord-Pas de Calais Region and Voies Navigables de France, FEDER funds through French research program: Programme de Recherche Concert es (PRC "Sites et Sols Pollu s, Environnement et Activit Humaines").

References

Bert V, Bonnin I, Saumitou Laprade P, de Laguerie P and Petit D, 2002. New Phytol. 155 (1): 47-57.

Cosio C, DeSantis L, Frey B, Diallo S, and Keller C, 2005. J. Exp. Bot. 56(412): 765-775.

Nolan NL, Zhang H, McLaughlin, 2005. J. Environ. Qual. 34: 496-507.

Sarret G, Saumitou-Laprade P, Bert V, Proux O, Hazemann JL, Traverse A, Marcus MA, and Manceau A, 2002. Plant Physiol. 130: 1815-1826.

Zhao FJ, Jiang RF, Dunham SJ, McGrath SP, 2006. New Phytol. 172: 646-654.