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# Is phytoextraction a suitable green treatment for metal-contaminated sediments?

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**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. These materials are usually spread on landfill sites. Phytoremediation could be a strategy for the reclamation of these polluted sediments. To our knowledge, phytoextraction with hyperaccumulating plants has been few tested on contaminated sediment. 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.

**Keywords:** *Arabidopsis halleri*, cadmium (Cd), hyperaccumulation, phytoextraction, sediment, speciation.

## Introduction

In the Northern part of France, the maintenance of waterways by regular dredging generates large volumes of metal-polluted sediments, which are often rich in metal contaminants and present a risk to the local environment. These materials are usually deposited on landfill sites (180 sites in the Nord-Pas de Calais region). The use of metal hyperaccumulating higher plants to extract metals accumulated in polluted soils (phytoextraction) might represent an alternative low cost remediation strategy [1, 2]. However, to our knowledge, there are few investigations into phytoextraction using hyperaccumulating plants on contaminated sediments [3, 4, 5, 6].

Plants called hyperaccumulators are defined by their ability to accumulate very high amount of metals in their aerial parts: more than 100, 1000 and 10000 mg kg<sup>-1</sup> dry weight (DW) for Cd, Ni and Zn, respectively [7, 8]. Second to this definition, *A. halleri* is considered as a hyperaccumulator, and, as a consequence, might be used in phytoextraction. Previous studies have shown that metal tolerance and accumulation traits vary as a function of the plant origin [9, 10]. In recent years, *Arabidopsis halleri* has been intensively studied for the genetics and physiology of metal tolerance and hyperaccumulation [5, 11, 12, 13, 14, 15, 16]. Moreover, this species develops populations on contaminated and uncontaminated soil [9, 17]. Despite these numerous studies, the mechanisms of cadmium accumulation in plants are still poorly understood. Besides, data concerning plant impact on Cd remaining in the substrate are lacking. Metal uptake by plants is often limited by low metal bioavailability in soil [18]. This parameter is mostly controlled by the pH and the composition of the soil (organic and amorphous oxides content, etc...) [19, 20, 21].

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<sup>1</sup> Equal participation

In this study, a 5-month pot experiment was conducted on a Zn- and Cd-contaminated dredged sediment with *A. halleri* of two origins, metallicolous and non-metallicolous. The sediment in its initial state was characterized and particularly the chemical form(s) of cadmium was determined using Scanning Electron Microscopy / Energy Dispersive Spectroscopy (SEM-EDS), micro X-Ray Fluorescence ( $\mu$ XRF) and, X-ray Absorption Fine Structure (XAFS) spectroscopy. Because Cd concentration is generally very low in the sediment, the sensitivity and micrometric resolution of synchrotron techniques are appropriate tools to identify and quantify Cd chemical species in heterogeneous systems. During the culture, plant growth parameters and metal accumulation were monitored. Metal concentrations and pH in the substrate and leachates as well as metal phytoavailability were also studied.

## Materials and methods

**Origin of the sediment.** The sampling area is located in the North of France, about 30 km south of Lille. This region is among the oldest and largest industrial areas in Europe. Past and present contamination by non-ferrous metal processing has released huge amounts of ore particles and slag into the environment [22]. Five hundred kg of sediment were collected by dredging the Scarpe River in an area known to be heavily polluted with Cd (around  $140 \text{ mg kg}^{-1}$ ). The sediment was mixed and stored for draining under cover for seven months in order to reduce its water content. Thereafter, the sediment was mechanically homogenized several times. Macroscopic characteristics of the bulk sediment including granulometry, physico-chemical parameters and major and trace element concentrations were determined.

**Plant material.** *A. halleri* develop natural populations on both metal contaminated and uncontaminated soils in Europe [9]. Seeds of *A. halleri* were collected in a smelter-impacted site (-Auby, Fr, pH= 6.80) and in an uncontaminated site (Hautes Fagnes, Be, pH = 6.94)

**Pot experiment.** *A. halleri* seeds were germinated on compost for 4 weeks. Seedlings were transferred to the sediment pots after roots had been washed carefully with deionised water. Seedlings were grown in 4 L pots containing 2,8 kg of sieved ( $<5\text{mm}$ ) sediment (Photo 1). Non vegetated pots were used as control. Each pot contained one seedling and was triplicated. Soil and sediment moisture was maintained at 80% field water capacity by regular addition of deionised water. Plants were grown for five months in controlled conditions (12h photo period, 20/16°C day/night temperature, 80% relative humidity – photo 1).

Before the start of the experiment, total metal concentrations, total carbon and pH in the sediment were measured. Health status and plant growth were regularly monitored by counting new leaves and measuring the size of the rosette. Once a month, leachates were collected at the bottom of the pots after the substrate (cultivated and non-cultivated) had been sprinkled with 1 L of deionised water. Besides, on monthly sacrificed pots, three plants were harvested to analyze their aboveground parts; Cd and Zn phytoavailability in sediment was performed using diffusive gradient in thin film (DGT) probes [23] and  $\text{Ca}(\text{NO}_3)_2$  extraction. Total Cd and Zn concentration were measured in sediment, leachates and plants.

**Chemical analyses.** Sediment samples were oven-dried at 50°C, ground and sieved ( $<100 \mu\text{m}$ ). Aliquots of 0,5 g of sediment was digested in close vessels using a mixture of HCl (6ml),  $\text{HNO}_3$  (2 ml) and  $\text{H}_2\text{O}$  (1ml) heated in a microwave oven. The filtered extract was adjusted to 100 ml with Milli-Q® water, and stored at 4°C before analysis. Leachates were filtered ( $0.45 \mu\text{m}$ ), then aliquots of 10 mL were acidified with  $\text{HNO}_3$  (suprapur®) and stored at 4°C before analysis.

At harvest, shoots and leaves were washed with deionised water. Leaves were oven-dried at 50°C for 3 days, then leaf biomass was weighed and finally ground and homogenised to prepare one aliquot per pot. Total concentrations of Cd and Zn in plant tissues were determined after digestion

of 1 g dry matter in 10 ml of HNO<sub>3</sub> and 3 ml of H<sub>2</sub>O, in a microwave oven. Solutions were filtered, adjusted to 100 ml with Milli-Q® water and stored at 4°C before analysis.

Metal concentrations in sediment, leachates and plant aboveground parts of *A. halleri* of two origins were determined by ICP-AES (inductively coupled plasma - atomic emission spectroscopy). Quality control for sediment and plant samples was based on the use of certified standard samples (sediment : NWRI TH-2 ; spinach leaves : SRM 1570a ) and internal control samples.

**Statistics.** Statistic analysis was performed using one-way ANOVA followed by post hoc comparison using Tukey's test for comparison of individual means, using the statistical program SPSS 15.0 (2007).

**Physical methods.** Cd speciation was studied in the sediment before the phytoextraction treatment. The sediment was freeze-dried, ground and pressed as pellets for EXAFS measurements. Cd K-edge EXAFS spectra of powder samples were recorded on the beamline FAME (ESRF, Grenoble, France) at 15K using a He cryostat. One part of the freeze-dried sediment was embedded in epoxy resin, cut to 30 µm-thick thin sections, micro-polished and mounted on glass slides. These sections were examined by environmental SEM-EDS and by µXRF, Cd L<sub>III</sub>-edge µXANES and S K-edge µXANES spectroscopy on the beamline LUCIA (SLS, Villigen, Switzerland) with a spot of 3x3 µm. Sample spectra were compared to model compound spectra, including Cd minerals, Cd-sorbed minerals and Cd-complexed organic compounds, fitted by linear combinations of these reference spectra and shell simulated using IFEFFIT suite of programs.

## Results & Discussion

### Sediment characterisation.

The sediment under study presents a neutral pH ( $7.48 \pm 0.01$ ) and is relatively rich in organic carbon ( $11.65 \pm 0.05$ ). After seven months, its water content is still of about 20 %. Cd and Zn contents are around 140 and 4000 DW mg.kg<sup>-1</sup>, respectively (table 1), *i.e.*, clearly higher than the geochemical background (around 0.5 and 75 mg.kg<sup>-1</sup> respectively, data from Agence de l'Eau Artois-picardie). Moreover, fine particles represent about 70 % of total weight sediment and Cd is mainly associated with these fine particles [24].

Table 1. Sediment metal concentrations [mg.kg<sup>-1</sup>], average on ten samples

	As	Cd	Cu	Pb	Zn
metal concentrations [mg.kg <sup>-1</sup> ]	57	141	233	600	4074
standard deviations	4	24	9	2	280

The speciation of Cd in the sediment before culture was examined by EXAFS spectroscopy. Linear combination fits of the sediment spectrum showed that greenockite was the closest reference, but did not match correctly the sediment spectrum. Adding a second component did not improve the fit, suggesting that our database of Cd reference compounds did not contain the species present in the sediment.

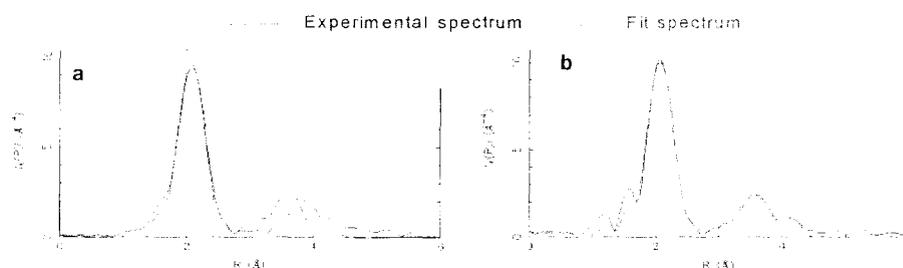


Figure 1: (a) Cd K-edge EXAFS spectrum for the bulk sediment and its Fourier transform (blue line) and FEFF simulation with a S first shell and a Cd second shell (red line). (b) Same spectrum simulated with a S first shell and a Cd, Zn, Fe second shell.

The sediment spectrum was then treated by FEFF simulations. The first shell was simulated with 3.9 S at 2.52 Å. The second shell was not correctly simulated with Cd only (Fig. 1a), but by a mixture of Cd and Zn (not shown) or of Zn, Cd and Fe (7.2 Zn, 2.6 Cd and 1.4 Fe at 2.51, 3.88 Å, 4.18 Å and 3.69 Å, respectively, Fig. 1b). This suggests that Cd occurs as a mixed Cd,Zn,Fe-sulphide. This result is consistent with the identification of pyrite (FeS) and sphalerite (ZnS) in similar sediments [25, 26]. CdS was also identified in marine sediments [27].

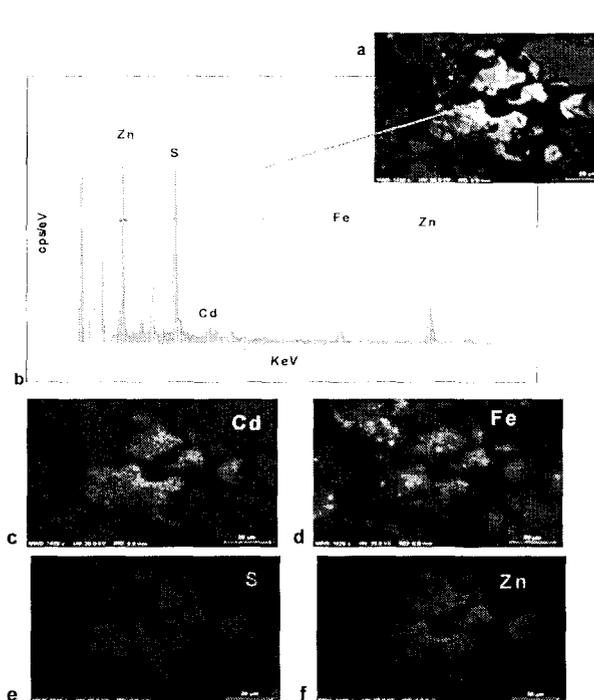


Figure 2: (a) backscattered electron SEM image and (b) EDS spectrum of a Cd-rich spot of the sediment thin section. (c-f) Elemental maps for Cd, Fe, S and Zn of this region.

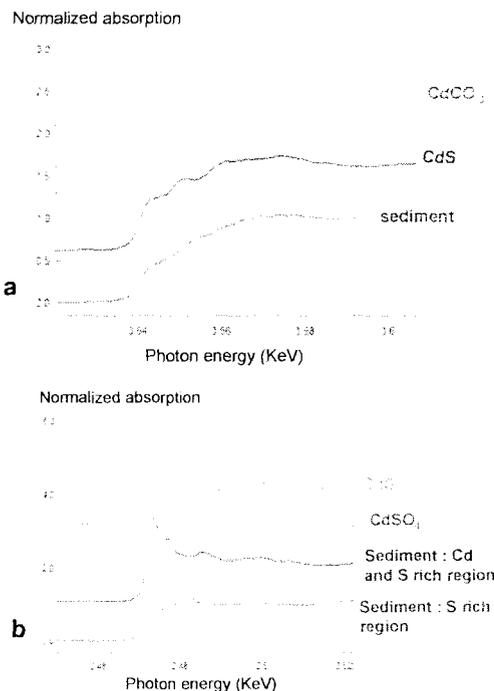


Figure 3: (a) Cd L<sub>111</sub>-edge μXANES spectra of a Cd-rich region of sediment and reference compounds and (b) S K-edge μXANES spectra recorded on two sediment samples (one Cd and S rich region and one S rich region)

Micro-scale investigations were achieved to precise Cd speciation and identify possible minor forms of Cd. SEM-EDS enabled the localization of Cd-rich regions in sediment thin sections and provided geochemical associations between the elements. The grains on the backscattered electron image shown in Fig. 2a contain associations of Cd, Fe, Zn and S (Fig. 2c-f), which is consistent with the EXAFS results.

The comparison of  $\mu$ XANES spectra recorded on Cd-rich regions with reference spectra supports the hypothesis of metallic sulphide as major Cd species (Fig. 3). Sulphur K-edge XANES spectroscopy is sensitive to the various forms of sulphur, whose oxidation state ranges from -II to +VI. Sulphur is present in reduced form in the metals- and S- rich grains, and as a mixture of reduced and oxidized species in other regions, (Fig. 3b).

To sum up, Cd was present as a mixed Cd,Zn,Fe-sulphide in the sediment. The sediment after the 5-month culture with *A. halleri* is currently being studied. Panfili et al. [25] showed that plants favour the oxidation of ZnS and the formation of Zn-phosphate, so a similar phenomenon might take place for Cd. Open questions include the stability of the Cd,Zn,Fe-sulphide and the nature of the distribution of a possible secondary species. These processes may change the bioavailability of Cd, and thus the efficiency of the phytoextraction.

### Plant growth parameters

Recall the experiment design, health of plants, rosette sizes and biomass were measured each month. Each pot contained one plant. Each month, three different individuals were measured that means that one plant is not monitored during the experiment.

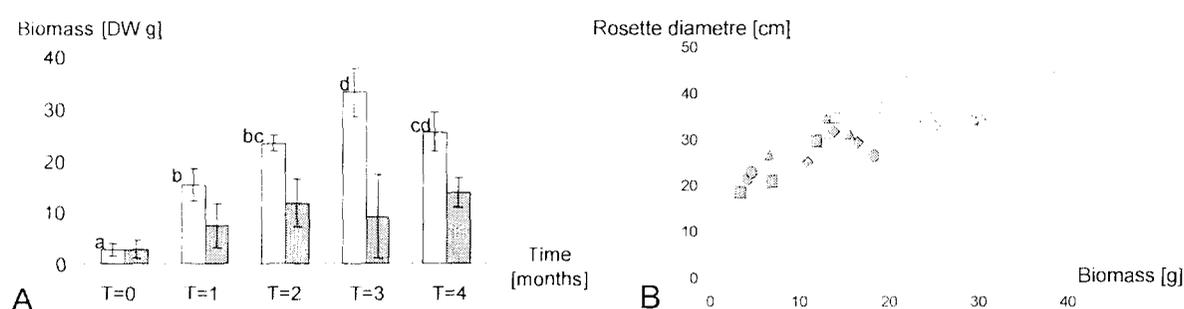


Figure 4 : (A) Average values of biomass ( $n=3 \pm$  SD) measured in aboveground part of *A. halleri* from a metallicolous (white) and non-metallicolous origins (dark grey) before plant transfer on sediment (T=0) and during the first four months of monitoring (T=1, T=2, T=3 and T=4). Letter means a significant difference at 5% (oneway ANOVA followed by a Tukey test). The oneway ANOVA is not significant for non metallicolous plants. (B) Relationship between plant biomass and rosette diameter for metallicolous (white) and non-metallicolous plants (dark grey) at each time of monitoring (rectangles represent T=1, triangles T=2, circles T=3 and diamond T=4).

At the beginning of the experiment, when plants were transferred on sediment, plants of both origins presented similar biomass, *i.e.*  $3.0 \pm 1.1$  g and  $3.2 \pm 1.7$  g (average for  $n=3 \pm$  standard deviation (SD), Fig.4-A). For metallicolous origin, aboveground part biomass was increased after one month, up to  $15.3 \pm 3.1$  g (Fig. 4-A). The increase of the biomass production for plants of non-metallicolous origin ( $7.4 \pm 4.3$  g at one month, Fig. 4-A) was not significant. Standard deviations were important; for instance after one month of growth, one plant produced 3.4 g of biomass whereas another plant produced 11.9 g of biomass. Regardless of the origin of the plants, plants looked healthy with no visual symptoms of toxicity..

From T1 to T4, plants from metallicolous origin produced more biomass than plants from non-metallicolous origin (Fig.4-A). The maximum difference in biomass is observed the third month of the experiment where plants of metallicolous origin reached  $33.1 \pm 4.6$  g whereas plants of non-metallicolous origin reached the average value of  $9.1 \pm 8.0$  g. Here again standard deviations for non-metallicolous origin were important..No significant increase in biomass was observed for plants from non-metallicolous origin between the first and fourth month (Fig. 4-A;  $p>0.05$ ). This difference between the two origins of *A. halleri* was highlighted by the correlation diagram between plant biomass and rosette diameter (Fig.4-B). Indeed, the two origins appeared clearly in two distinct groups, with higher biomass and rosette diameter for metallicolous plants (13 to 38.5 g and 29.5 to 44.5 cm) than for non-metallicolous plants (3.4 to 18.4 g and 18.5 to 34.5 cm).

From biomass results, *A. halleri* from metallicolous origin appeared more suitable for phytoextraction than *A. halleri* from non-metallicolous origin. Because of the high interindividual variability (this work was not done with clones), it was difficult to conclude about the tolerance of the two studied origins of *A. halleri*.

### Evolution of Cd and Zn concentrations in aboveground parts of *A. halleri*

The average of Cd concentrations were measured each month on different plant (Fig.5-A). It meant twelve values of Cd concentration for each population of *A. halleri*. For each origin tested separately, no significant difference was observed ( $p>0.05$ ) (Fig. 5-A). Maximum average Cd concentrations were  $145 \pm 90 \text{ mg.kg}^{-1}$  ( $n=3 \pm \text{SD}$ ) and  $185 \pm 18 \text{ mg.kg}^{-1}$  for, respectively, metallicolous origin at T4 and non-metallicolous origin at T1 (Fig.5-A). At T4, the Cd concentration non metallicolous plants was  $129 \pm 40 \text{ mg.kg}^{-1}$  ( $n=3 \pm \text{SD}$ ). There was not significant difference between origins ( $p>0,05$ ). Standard deviations were important, suggesting a high interindividual variability in Cd accumulation.

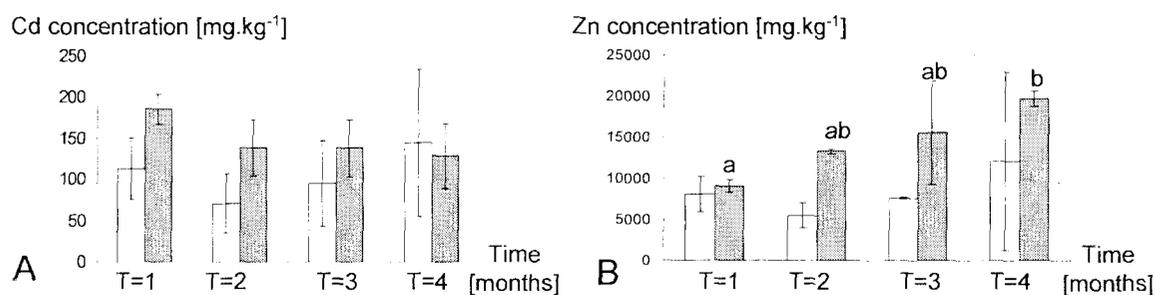


Figure 5 : Average of Cd (A) and Zn (B) concentrations [mg.kg<sup>-1</sup>] measured in aboveground parts of *A. halleri* from metallicolous (white) and non-metallicolous (dark grey) origins during the first four months of monitoring (averages and standard deviations over 3 samples).

Fig. 5 B: A letter means a significant difference at 5% (oneway ANOVA followed by a Tukey test). The oneway ANOVA is not significant for metallicolous plants.

To sum up, Cd concentrations did not increase significantly with time regardless the origin of *A. halleri*. Plants seemed to have reached a maximum value after one month only. This species, growing in hydroponic solutions reached Cd concentration in their leaves about  $6000 \text{ mg.kg}^{-1}$  after 14 weeks [16]. In order to explain this “plateau response” two hypotheses can be proposed. Cd accumulation could be limited by Cd phytoavailability [18, 28, 29, 30] or the plant itself could limit the entry of the metal to prevent toxicity [9, 31, 32].

For Zn accumulation, *A. halleri* from metallicolous origin showed the same behaviour than for Cd accumulation. For metallicolous plants, no significant difference was observed at the various times ( $p>0.05$ ) (Fig. 5-B). Again, standard deviations were very high, suggesting a high individual variability of plants, particularly at T4. Indeed, the three replicate plants had accumulated 24577, 4578 and 7091  $\text{mg.kg}^{-1}$  (Fig. 5-B). On the contrary, Zn concentration in aboveground parts of *A. halleri* from non-metallicolous origin increased significantly between the first and the four month of culture. At T2 and T3, plants from non-metallicolous origin accumulated more Zn than plants from metallicolous origin (Fig. 5-B), but interindividual variability was important at T3 (22835, 12281 and 11578  $\text{mg.kg}^{-1}$ ). To conclude on Zn accumulation, *A. halleri* from non-metallicolous origin show a different behaviour than for Cd accumulation. This result is consistent with that of Bert et al. [17].

### Phytoextraction yield of *Arabidopsis halleri*.

From results of biomass, Cd and Zn accumulation in aerial part, in leachates and in sediment, the amount Cd present in the sediment and extracted by *A. halleri* was calculated. Put under optimal conditions in the growth chamber, for both origins, after 1-month growth, plants

extracted around 1.5 mg pot<sup>-1</sup>. On the other hand, after 4-month growth, plants from metallicolous origin extracted about 3.7 ± 0.3 mg pot<sup>-1</sup> whereas plant from non-metallicolous extracted 1.8 ± 0.1 mg. For plants from metallicolous origin, the extraction yield seemed to increase with time (table 2). Indeed, after one month of growth, the plant extracted 0.41 ± 0.1 % of the Cd present in the sediment pot, and 0.98 ± 0.7 % after four months (table 2). The maximum Cd extraction was 1.8 % and was obtained after four months. By contrast, Cd extraction by *A. halleri* from non-metallicolous origin were significantly lower (0.7 % maximum, obtained after three and four months). For both origins, *A. halleri* extracted more Zn than Cd in their aboveground parts. The maximum Zn extraction yield was 6.3 % after three month for the metallicolous origin, and 2.9, % after four months of growth for the other origin.

Less than 0.05 % of Cd present in the sediment pot at the beginning of the experiment was lost in leachates. In spite of the Cd extraction by *A. halleri* and Cd lost in leachates, the Cd majority still in the sediment (table 2). The same trends were observed for Zn (table 2).

Table 2 : Material balance of Cd, in percent, between plant, leachates and sediment compartments, after one and four months of culture by *A. halleri* from a metallicolous and a non-metallicolous origin.

<i>A. halleri</i> origin	culture time [months]	Extracted by plant [%]		in leachates [%]		still in sediment [%]	
		Cd	Zn	Cd	Zn	Cd	Zn
metallicolous	1	0,41 ± 0,10	1.03 ± 0.2	0,025 ± 0.010	0.05 ± 0.02	88,0 ± 5.0	93.34 ± 5.48
non metallicolous		0,34 ± 0,17	0.63 ± 0.4	0,025 ± 0.012	0.04 ± 0.02	90,1 ± 2,1	100.45 ± 1.90
metallicolous	4	0.98 ± 0,74	3.08 ± 3.0	0,044 ± 0,020	0.05 ± 0.03	96,7 ± 2.9	92.33 ± 1.49
non metallicolous		0.46 ± 0,21	2.52 ± 0.6	0,019 ± 0,010	0.02 ± 0.01	91,6 ± 2.2	91.06 ± 3.38

## Conclusion et perspectives

Second Cd and Zn concentrations in leaves, *A. halleri* regardless the plant origin, was really a Cd and Zn hyperaccumulator [7]. For phytoremediation, the ideal plant should possess multiple characteristics including fast growing, high biomass, deep roots, easeto harvest and high metal tolerance and accumulation in their aerial part and harvestable parts [33]. Klang-Westin and Eriksson [34] postulated that biomass production is the crucial parameter for phytoextraction. In our study, the biomass production and metal phytoextraction was higher for the metallicolous origin. But the success of phytoextraction also depends on the substrate [35]. A very precise characterization of the substrate and Cd chemical form and phytoavailability is needed. These aspects are being currently studied.

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