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Accumulation Forms of Zn and Pb in *Phaseolus vulgaris* in the Presence and Absence of EDTA

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The internalized speciation of Zn and Pb in roots and leaves of *Phaseolus vulgaris* grown in zinc sulfate, zinc EDTA, lead nitrate, and lead EDTA solutions were studied by electron microscopy (Zn) and extended X-ray absorption fine structure (EXAFS) spectroscopy (Zn and Pb). Zn was predominantly present as Zn phosphate dihydrate in the roots and leaves of the plant regardless of its form in solution. Pb was predominantly found in the leaves as cerussite (lead carbonate) when the plant was grown in Pb nitrate solution and as a mixture of PbEDTA and an undetermined species in contact with PbEDTA solution. Therefore, *Phaseolus vulgaris* is able to dissociate totally (Zn) or partly (Pb) the two metal−EDTA complexes from the nutrient solution and to bind these metals in other forms.

Introduction

Phytoextraction, an emerging biotechnology that uses plants to transfer trace elements from polluted soils to plant shoots, is both technically and economically attractive in comparison to traditional civil-engineering practices involving intensive soil manipulation. Initial phytoremediation studies focused on hyperaccumulating species such as *Thlaspi caerulescens* (*1*−*3*), *Thlaspi rotondifolium* (*4*), and *Alyssum lesbiacum* (*5*) which can grow in extremely polluted soils and can accumulate up to several percent metal in their shoots. However, a general characteristic of these species is their slow growth and limited biomass production. Therefore, some researchers estimated that these species could not meet the requirements for the design of an economically realistic biotechnology, which are, in the case of Pb, 1% metal concentration in shoots and 20t ha^{-1} $year^{-1}$ shoot biomass productivity (*6*). For this reason, more recent research on phytoextraction focused on crop species such as Indian mustard, corn, oat, barley, pea, and ryegrass that display significant heavy metal tolerance combined with a high biomass productivity (*6*−*13*). To compensate for the relatively low metal accumulation capacities of these species, some researchers proposed to favor the soil/root transfer of metals by adding chelates such as EDTA, DTPA, CDTA, EGTA, or citric acid, which favor metal desorption from minerals (*6, 8, 9, 11*−*14*). Among these chelates, EDTA was shown to be the most efficient, as Pb shoot concentrations increased from less than 100 mg/Kg to 1.5% for Indian mustard grown in soils containing 600 mg/Kg Pb (*8*) and from less than 500 mg/Kg to 2% for corn grown in soils containing 2500 mg/Kg Pb (*9*).

EDTA has been shown not only to enhance Pb desorption from the soil components to the soil solution but also to increase its transport into the xylem and its transfer from the roots to the shoots (*9, 14*). Several studies on Pb accumulation in plants in the presence of ^{14}C-labeled EDTA showed that both Pb and EDTA were present in the shoots, suggesting that the metal was absorbed and transferred as a PbEDTA complex (*8, 14, 15*). The physiological basis of the uptake of the complex and particularly the possibility for this negatively charged large molecule to cross the membrane is unknown. However, Vassil et al. (*15*) suggested that EDTA could damage the membrane of root cells by chelating Zn^{2+} and Ca^{2+} cations that stabilize this membrane, thus allowing free equilibration between the soil solution and the xylem sap. However, no evidence has been found that favors the hypothesis of an uptake of the $PbEDTA^{2-}$ complex or of Pb^{2+} and $EDTA^{4-}$ separately.

To our knowledge, no studies have been conducted on Zn and EDTA phytoaccumulation, but given the high affinity of this metal for EDTA ($\log K = 16.44$ for $ZnEDTA^{2-}$ compared to 17.88 for $PbEDTA^{2-}$ (*16*)), the chelate is expected to have a positive effect on Zn phytoextraction. Despite its effectiveness in phytoextraction, the use of EDTA in the environment is subject to discussion because of its low biodegradability.

Different mechanisms of Zn accumulation in the plant tissues have been proposed depending on the plant species studied (*17*). Brookes et al. (*18*) showed that zinc-resistant clones of *Deschampsia caespitosa* were able to actively pump zinc into the vacuoles of root cells, whereas zinc-sensitive clones had a much lower capacity to do so. Some Zn-containing granules have been identified by transmission electron microscopy and energy-dispersive X-ray analysis (TEM-EDX) in small vacuoles of root cells of various plants species (*Deschampsia caespitosa* and some crop species) grown in Zn solutions (*19*−*22*). Based on the presence of P, Mg, and K in these globular deposits and on the relatively constant Zn/P, Mg/P, and K/P elemental net count ratios (0.29−0.48, 0.32−0.41, and 0.39−0.43, respectively) the authors concluded that they contained Zn phytate, a *myo*-inositol *kis*-hexaphosphate. Phytate is a molecule present in the cells of grains, seeds, and plants, generally present as Ca, Mg salt and associated with carbohydrates and proteins to form globular bodies, and whose main function is P, Mg, and K storage (*23*). Phytate presents a high affinity for Zn and Fe and could act as a metal-immobilizing molecule as well (*24*). Vazquez et al. (*25*) identified by TEM-EDX some Zn-containing globular crystals in vacuoles of *Thlaspi caerulescens* grown in Zn-containing nutrient solutions but with higher Zn/P elemental net count ratios (up to 6.9 in leaves and 8.8 in roots) and no Mg. They concluded that this Zn/P ratio was incompatible with Zn phosphate (Zn/P = 1.5) and Zn phytate composition (Zn/P = 0.33 for Zn_2-phytate and 0.17 for $ZnNa_{10}$-phytate). Other chemical forms of Zn were

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TABLE 1. Calculated Speciation of Zn and Pb in the Nutrient Solutions

nutrient solution	composition[a]	metal speciation
ZnEDTA	Hoagland + 50 μM $ZnSO_4$ + 50 μM Na_2H_2EDTA	96% $ZnEDTA^{2-}$, 4% Zn^{2+}
Zn sulfate	Hoagland + 50 μM $ZnSO_4$	97% Zn^{2+}, 3% $ZnSO_4$
PbEDTA	Hoagland + 125 μM $Pb(NO_3)_2$ + 125 μM Na_2H_2EDTA	100% $PbEDTA^{2-}$
Pb nitrate	tap water + 1666 μM $Pb(NO_3)_2$	80% Pb^{2+}, 14% $PbSO_4PbO$, 3% $PbCl^+$, 3% $PbNO_3^+$

[a] The pH was fixed at 5.5 for the four solutions.

identified by X-ray absorption spectroscopy in the same plant species also grown in Zn-containing nutrient solution. Salt et al. (*26*) found that Zn was mostly complexed to histidine in roots, transported as Zn^{2+} in the xylem sap, and complexed to organic acids in leaves.

In the case of Pb, some authors suggested that cell walls could play an important role in the accumulation of metals (*17*). This hypothesis has been verified by EXAFS spectroscopy in the case of the lichen *Xanthoria parietina* (*27*) and by TEM-EDX and EXAFS spectroscopy in the case of the heavy metal tolerant grass *Agrostis capillaris* (*28*). This latter species presented extracellular Pb-containing grains in the outermost layer of root cells predominantly composed of pyromorphite, a lead phosphate mineral.

The S-containing proteins, phytochelatins, have been often advocated to complex metals in plants, but this hypothesis has not been demonstrated yet for Zn and Pb.

In this paper, EXAFS spectroscopy and TEM-EDX were used to investigate the accumulation forms of Zn and Pb in bean (*Phaseolus vulgaris*) grown in metal-containing solutions, in the presence and absence of EDTA.

Materials and Methods

Materials. Bean seeds (*Phaseolus vulgaris* L. cv. Limburgse vroege) received a cold treatment (+4 °C) for 3 days to break dormancy and to synchronize germination. They were transferred to a growth chamber to germinate between two layers of water-soaked rock wool for 4 days. Subsequently, seed coats were removed, and seedlings with a root length of approximately 1.5 cm were grown in 3-mm thick polystyrene squares by fixing the roots through 5-mm holes (9 plants/treatment). The polystyrene was floated on 3 L of aerated Hoagland's solution in 3.5 L polyethylene beakers. Plants were grown under controlled conditions (12 h photoperiod, PAR = 165 μmol m^{-2} s^{-1} at leaf level, 65% RH, 22 °C). Unless otherwise stated, plants were grown in solutions containing 1.0 mmol/L K^+, 0.3 mmol/L Ca^{2+}, 0.2 mmol/L $H_2PO_4^-$, 0.2 mmol/L Mg^{2+}, 0.2 mmol/L NH_4^+, 0.6 mmol/L NO_3^{2-}, 0.2 mmol/L SO_4^{2-}, 1.8 μmol/L Cl^-, 4.6 μmol/L H_3BO_3, 0.05 μmol/L MoO_3, 0.9 μmol/L Mn^{2+}, 1.6 μmol/L Fe^{2+}, 0.08 μmol/L Zn^{2+}, and 0.03 μmol/L Cu^{2+}, whose pH was fixed at 5.5 with HCl. Conditions of metal exposure were chosen based on plant responses, to obtain similar growth inhibitions. For the Zn experiments, Zn alone (50 μmol/L $ZnSO_4$) or equivalent amounts of Zn and EDTA (50 μmol/L $ZnSO_4$ and Na_2H_2EDTA) were added to the nutrient solution after 1 week of growth, and samples were collected after 72 h of Zn exposure. For the PbEDTA experiment, 125 μmol/L $Pb(NO_3)_2$ and 125 μmol/L Na_2H_2EDTA were added from the beginning of the hydroponic culture, and plants were grown for 2 weeks. For the EDTA-free experiment, the plants were grown for 2 weeks in tap water at pH 5.5, to which 1666 μmol/L $Pb(NO_3)_2$ was added at the beginning of the culture. Tap water was used instead of a nutrient solution in order to avoid Pb precipitation. Its composition is as follows: 0.07 mmol/L K^+, 1.51 mmol/L Ca^{2+}, 0.22 mmol/L Mg^{2+}, 0.03 mmol/L NO_3^{2-}, 0.13 mmol/L SO_4^{2-}, 1.32 μmol/L Cl^-, 0.46 μmol/L Zn^{2+}, 0.14 μmol/L Ba^{2+}, and 1.47 μmol/L Sr^{2+}. Cu^{2+}, $H_2PO_4^-$, and NH_4^+ concentrations were below 0.16, 1.5, and 7.7 μmol/L, respectively. A control series was grown in Hoagland's medium. For all the cultures, the nutrient solutions were renewed every 24 h in order to keep the chemical composition stable during the growth. After 24 h, the pH of the nutrient solutions was still 5.5. Metal concentrations in the plants were determined by ICP-AES after digestion in aqua regia. The values given in this paper correspond to the mean concentration over 3−6 samples ± SD. Zn and Pb speciation in the nutrient solutions were calculated using MINTEQA2 (Table 1).

TEM-EDX. Fixation of leaf and root samples was carried out for 2 h at 4 °C in 4% glutaraldehyde. To test the possible redistribution or loss of metals, the fixation was done in the absence and in the presence of Na_2S (1% w/v) to precipitate the metals. Comparison indicated a loss of Zn only in the apoplast. Dehydration was performed in an ethanol series (20, 40, 60, 80, 94, and 100%). After 2 h in absolute alcohol, samples were embedded in Spurr's resin. Sections 0.5 μm thick were cut using a Leica Ultracut UCT ultramicrotome. TEM was performed on a Philips scanning transmission electron microscope (STEM, CM 12) equipped with an EDAX PV9900 energy-dispersive X-ray analyzer, with a super ultrathin window. The accelerating voltage was 120 kV for the imaging and 100 kV for the microanalysis. The electron beam size on the sample was 100−200 nm. Zn/P ratios were calculated using uncorrected net counts for the K_α peaks. EDX data were collected for 25 samples of Zn-treated plants and 10 control plants.

EXAFS. Roots and leaves of *Phaseolus vulgaris* were freeze-dried, ground, and pressed as 2 mm thick pellets for the EXAFS experiments. Zn K-edge and Pb L_{III}-edge EXAFS measurements were performed on the BM32 CRG/IF beamline at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) with a beam current of ~180 mA. Spectra were recorded at room temperature in fluorescence detection mode using a Canberra 30-element array germanium detector with a total counting rate of 600 000 photons/s. X-ray absorption spectra were treated following a standard procedure (*29*). Briefly, EXAFS spectra ($\chi(k)$) were obtained by subtracting a function $y = a + bx^n$ to the total absorption and dividing the difference by a normalizing two-range spline. The kinetic energy (E_k) of the photoelectron was converted to wavevector (k) value by taking the origin ($E_k = 0$) at the half-height of the edge. k^3-weighted (Zn) and k-weighted (Pb) EXAFS spectra were then compared to reference spectra. The likeness of the known and unknown spectra was quantified by the sum of the square differences $FM = \Sigma(k^3\chi_{known} - k^3\chi_{unknown})^2$ in the 2.0−9.5 $Å^{-1}$ range for Zn and by $FM = \Sigma(k\chi_{known} - k\chi_{unknown})^2$ for Pb. A $k = 1$ weighting was chosen for lead because a higher k value decreased the signal/noise ratio in the high k range, so the fitting program did not fit properly the low k region. Zn K-edge radial structure functions (RSFs) were obtained by Fourier transforming $k^3\chi(k)$ spectra using a Kaiser function window. Interatomic distances (R), number of atoms in the successive atomic shells (N), and the Debye−Waller factors (σ^2) were evaluated by least-squares fitting Fourier filtered EXAFS spectra. Amplitude and phase shift functions for Zn−O and Zn−P atomic pairs were

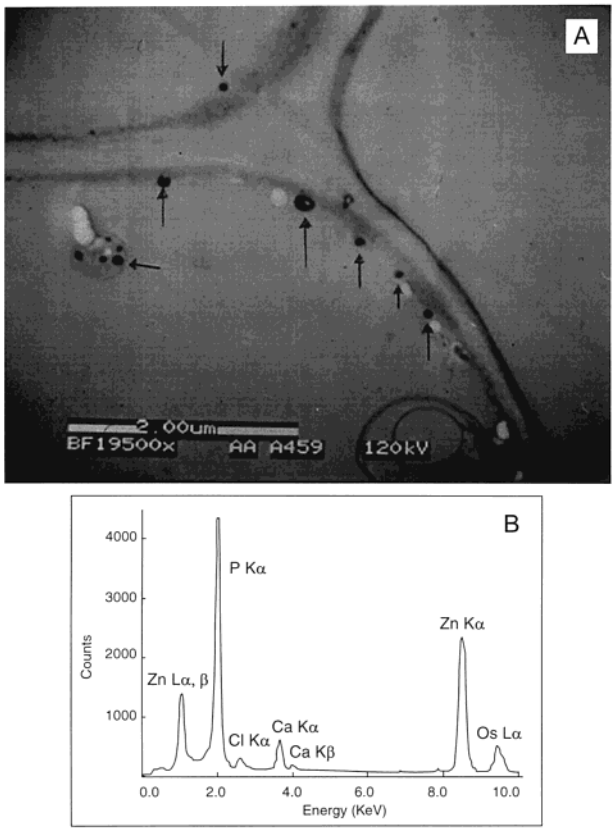

FIGURE 1. A. Transmission electron micrograph of leaves cells of *P. vulgaris* grown in Zn sulfate solution. The cells contain dense granules of about 0.1 microns in diameter near the membrane and in chloroplasts. B. Electron probe microanalysis spectrum of one of the granules shown on A. Accumulation rate: 4600 counts/s, acquisition time: 60 s.

calculated ab initio from the structure of hopeite ($Zn_3(PO_4)_2 \cdot 4H_2O$) (*30*) using FEFF7 code (*31*).

Results and Discussion

Metal Concentration. Measured Zn concentrations were 1468 ± 74 and 254 ± 20 mg/Kg dry weight in roots and leaves of the plants grown in Zn sulfate solution, respectively, and 503 ± 53 and 600 ± 39 mg/Kg in roots and leaves of the plants grown in ZnEDTA solution. These values suggest that Zn transfer from roots to shoots is increased in the presence of EDTA, which is in agreement with observations made on Pb accumulation (*9, 14*). Pb concentration in roots was not determined. Measured Pb concentrations in the leaves were 272 ± 25 mg/Kg for the plants grown in Pb nitrate solution and 1047 ± 40 mg/Kg for the plants grown in PbEDTA solution.

Transmission Electron Microscopy and X-ray Microanalysis. TEM observation of leaf cells for *P. vulgaris* exposed to 50 μM Zn solution revealed the presence of small particles ($<0.1\,\mu$m) in the cytoplasm and in some chloroplasts (arrows in Figure 1A). EDX analysis of these precipitates showed that they contain high amounts of Zn and P, a small amount of Ca, but no K (Figure 1B). The Zn/P net count ratio was relatively constant (0.5 ± 0.1). This value lies between values reported in the literature for other plant species (see Introduction). For the control plants, no granules were observed, and no Zn was detected. These results suggest that Zn is accumulated in the leaves of *P. vulgaris* as a phosphate compound, but one cannot conclude on the nature of the P-containing ligand. To obtain structural information on the Zn chemical form and to compare Zn and Pb speciation in *P. vulgaris*, leaves and roots samples were analyzed by EXAFS spectroscopy.

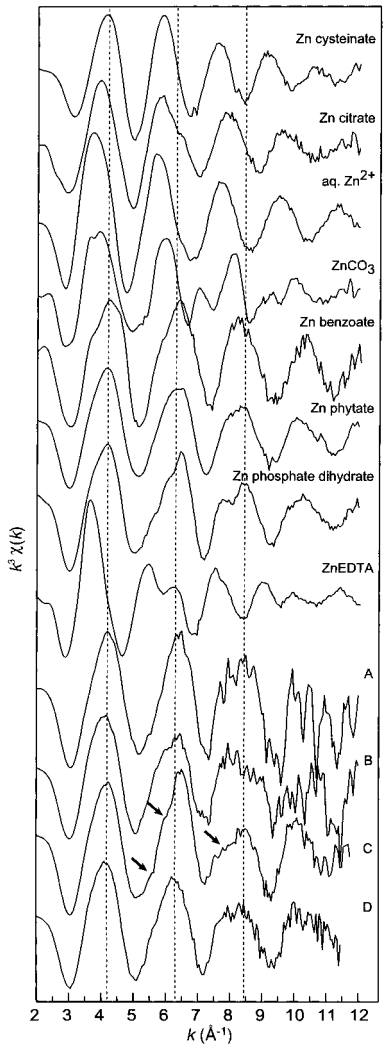

FIGURE 2. Comparison of Zn K-edge EXAFS spectra for *P. vulgaris* grown in ZnEDTA- (A: roots, B: leaves) and Zn sulfate- (C: roots, D: leaves) containing nutrient solution and for a selection of model compounds. The sample "aq. Zn^{2+}" corresponds to a solution containing 0.1 mol/L $Zn(NO_3)_2$ at pH 4, whose calculated Zn speciation is 100% Zn^{2+}.

EXAFS. Zn K-Edge. Four samples, including the roots and the leaves of *P. vulgaris* grown in ZnEDTA (samples A and B) and Zn sulfate media (samples C and D), were examined at the Zn K-edge. The spectra were compared to a database of mineral and organic Zn compounds (*32*), a selection of which is shown in Figure 2. The spectra for samples A and B are clearly different from the ZnEDTA model compound (FM = 2.42×10^3 and 1.43×10^3, respectively). In the nutrient solution containing Zn and EDTA, the calculated Zn speciation is 96% $ZnEDTA^{2-}$ and 4% Zn^{2+} (compared to 97% Zn^{2+} and 3% $ZnSO_4$ in the nutrient solution containing Zn sulfate). Thus, as suggested by TEM-EDX, EXAFS shows that the Zn chemical form has changed from the ZnEDTA solution to the plant. For those two samples, the closest spectra were Zn phosphate dihydrate (FM = 152 and 70, respectively), Zn phytate (FM = 152 and 79), and Zn benzoate (FM = 128 and 133). For samples C and D, these three model compounds were also the closest match (FM = 36 to 135). Sample C spectrum presents a great similarity with Zn phytate spectrum, particularly the asymmetric shape of the second oscillation and the presence of two shoulders on its left tail and the occurrence of a shoulder on the third oscillation

TABLE 2. Structural Parameters Determined by Simulating EXAFS Spectra for Zn in *Phaseolus vulgaris* Roots and Leaves[a]

	first O-shell			second P shell			
sample	R_O (Å)	N_O	σ^2 (Å^2)	R_P (Å)	N_P	σ^2 (Å^2)	Q
Zn phosphate dihydrate	1.97	4.0	0.008	3.16	2.0	0.008	0.012
				3.60	2.0	0.012	
Zn phytate	1.98	4.0	0.006	3.12	0.9	0.008	0.016
				3.60	0.6	0.012	
***Phaseolus vulgaris* Grown in ZnEDTA Solution**							
A, roots	1.95	3.9	0.005	3.13	1.6	0.005	0.012
				3.51	0.8	0.012	
B, leaves	1.99	4.0	0.010	3.12	1.0	0.004	0.022
				3.64	0.8	0.012	
***Phaseolus vulgaris* Grown in Zn Sulfate Solution**							
C, roots	1.96	3.9	0.006	3.14	1.2	0.005	0.021
				3.60	0.7	0.004	
D, leaves	1.99	4.0	0.008	3.15	0.9	0.004	0.015
				3.64	1.0	0.012	

[a] Q: figure of merit for the spectral fit. $Q = \Sigma(k^3\chi_{exp} - k^3\chi_{th})^2/\Sigma(k^3\chi_{exp})^2$ in the 3.6−11.4 Å^{-1} range. The precisions, calculated from a degradation of the fit to 2Q, are 0.01 Å for R_O, 0.03 Å for R_P, 20% for N, and 0.003 Å^2 for σ^2.

(arrows in Figure 2). The three references have the Zn first atomic shell in common, which is composed of four oxygen atoms (Table 2, (*33*)). The second shell is composed of phosphorus atoms in Zn phosphate dihydrate and Zn phytate and of carbon in Zn benzoate. As previously described (*34*), it is possible to distinguish P and C next nearest neighbors by comparing the imaginary part of the second peak of the Fourier transform spectra, which is shifted to the left for C atoms. The position of the imaginary function in the four plant spectra matched that in the Zn phosphate dihydrate and the Zn phytate (Inset in Figure 3). Moreover, the amplitude of the second RSF peak was higher for the plant samples than for Zn phytate. These observations tend to suggest that in the four plant samples, Zn is bound to inorganic phosphate groups (e.g. Zn phosphate) rather than organic (e.g. phytate).

The structural parameters for the Zn phosphate dihydrate and phytate references (whose crystallographic structures are not known) and the four plant samples were determined by quantitative analysis of the EXAFS spectra (Table 2). For both references, the first shell was simulated by a tetrahedral oxygen shell containing 4 ± 0.7 P atoms at 1.97 ± 0.01 and 1.98 ± 0.01 Å for Zn phosphate dihydrate and Zn phytate, respectively. The second shell was simulated by two subshells containing 4 ± 0.7 P atoms in Zn phosphate dihydrate and only 1.5 ± 0.5 P atoms in Zn phytate, which indicates that Zn is bound on average to less than four phosphate groups in this latter compound (Figure 4). The plant spectra were simulated by a first shell composed of oxygen with distances typical of tetrahedral coordination (1.96 ± 0.01 Å for the roots and 1.99 ± 0.01 Å for the leaves) and by a second shell composed of two P subshells containing 1.8 to 2.4 ± 0.7 P atoms (Table 2). The number of next nearest P atoms is higher than for Zn phytate, as expected from the comparison of the second RSF peaks (Figure 3). If Zn was present as Zn phytate in the plant samples, the number of atomic neighbors could be lower than in the reference because of lower crystallinity. Thus, it is concluded that Zn phosphate dihydrate is the major form of Zn in the four plant samples, whatever the form of Zn (Zn^{2+}, $ZnEDTA^{2-}$) in the growing solution.

Pb L_{III}-Edge. The fingerprint approach is particularly adapted to Pb L_{III}-edge spectra because this metal exhibits a large variety of local environments, thus important differences in frequency and amplitude are observed between

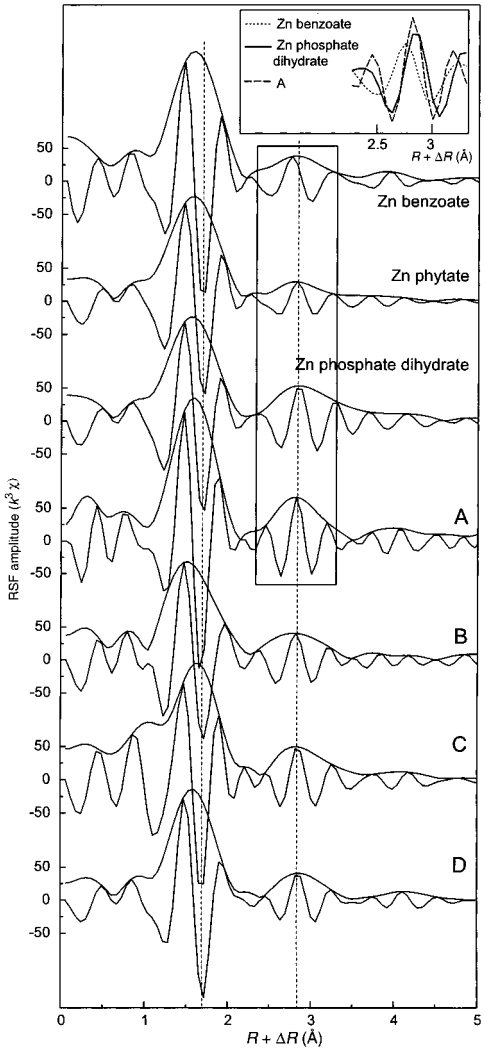

FIGURE 3. Radial structure functions (modulus and imaginary part) for *P. vulgaris* grown in ZnEDTA- (A: roots, B: leaves) and Zn sulfate-containing nutrient solution (C: roots, D: leaves), and for Zn benzoate, Zn phytate and Zn phosphate dihydrate. Inset: Zoom on the imaginary parts in the 2.3−3.3 Å range.

Pb reference spectra (*35, 36*). Pb L_{III}-edge EXAFS spectra of *P. vulgaris* leaves were compared to a database of mineral and organic Pb compound spectra (*32, 36*). For the leaves of the plant grown in the absence of EDTA (sample E), a very good spectral agreement was obtained with cerussite (anhydrous lead carbonate) (FM = 0.0165, Figure 5). Particularly, the shoulders on the third and fifth oscillations at 4.7 and 7.5 Å^{-1}, that constitute good fingerprints for $PbCO_3$, are observed on the "leaves" spectrum. However, this latter spectrum presents a slightly lower amplitude, which likely results from the contribution of minor Pb species. In an attempt to identify the minor species, the spectrum was simulated by a linear combination of two reference spectra. A combination of 85% cerussite and 15% pyromorphite (Figure 5) and a combination of 83% cerussite and 17% Pb benzoate (not shown) provided the same quality of fit (FM = 0.0043). Thus, it is concluded that the major form of Pb in leaves of *P. vulgaris* grown in Pb nitrate-containing solution is cerussite, but the nature of the minor species, whose proportion is lower than 20%, remains uncertain. Cerussite was absent from the nutrient solution, as shown by the calculated speciation of Pb in the tap water (80% Pb^{2+}, 14% larnakite ($PbSO_4PbO$), 3% $PbCl^+$, and 3% $PbNO_3^+$, Table 1). Thus, cerussite likely precipitated in the plant itself.

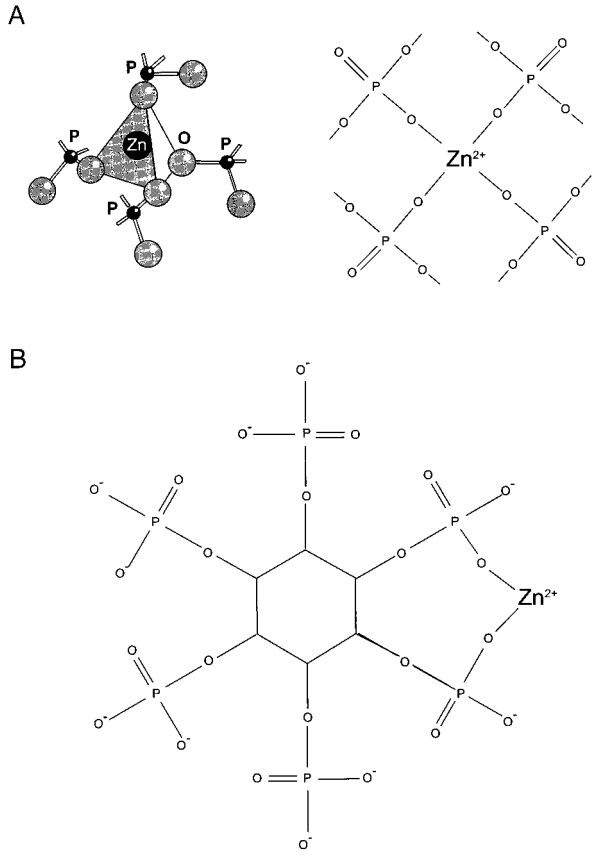

FIGURE 4. Possible Zn local structures for Zn phosphate dihydrate (A) and Zn phytate (B), where Zn is bound to four (A) and two (B) phosphate groups.

For the plant grown in the presence of PbEDTA (sample F), MINTEQA2 calculation showed that 100% of Pb is complexed to EDTA in the solution (Table 1). However, the spectra for sample F and for $PbEDTA^{2-}$ are clearly distinct. Also, spectrum F differs from spectrum E (Figure 5). The amplitude of spectrum F is particularly low, which is indicative of a distribution of species. Accordingly, no good agreement was obtained with one reference spectrum. The best statistical solution was obtained for a combination of 51% pyromorphite, 27% $PbEDTA^{2-}$, and 22% Pb salicylate (FM = $4.4\ 10^{-3}$, Figure 5), but satisfying solutions were also obtained with mixtures of cerussite, $PbEDTA^{2-}$, and various Pb-organic acid complexes. In all cases, $PbEDTA^{2-}$ systematically showed up, and, therefore, its presence is very likely. When $PbEDTA^{2-}$ was excluded from the fit, FM values increased to $7.0\ 10^{-3}$. Thus, Pb is probably present as a mixture of $PbEDTA^{2-}$ and other species that are difficult to identify because of the low amplitude of Pb-organic compounds EXAFS spectra. Thus, it can be concluded from these results that both Pb and EDTA can be absorbed by the plant and that one part of Pb present in the leaves is complexed to EDTA. The mechanism of $PbEDTA^{2-}$ or Pb^{2+} and $EDTA^{4-}$ absorption and transport through the membrane is still to be elucidated.

This study shows that the mechanism of metal accumulation in *P. vulgaris* depends on the nature of the metal studied. For Zn, no difference was observed between plants grown in ZnEDTA and Zn sulfate solution. In both cases, Zn predominantly precipitated as Zn phosphate dihydrate in the roots and in the leaves. In contrast, cerussite was the predominate Pb species in the absence of EDTA, but in the presence of EDTA, a mixture of $PbEDTA^{2-}$ and unidentified Pb species was the result. Thus, highly stable metal−EDTA complexes present in soil solutions can be totally (Zn), or partly (Pb), dissociated when absorbed by the plant.

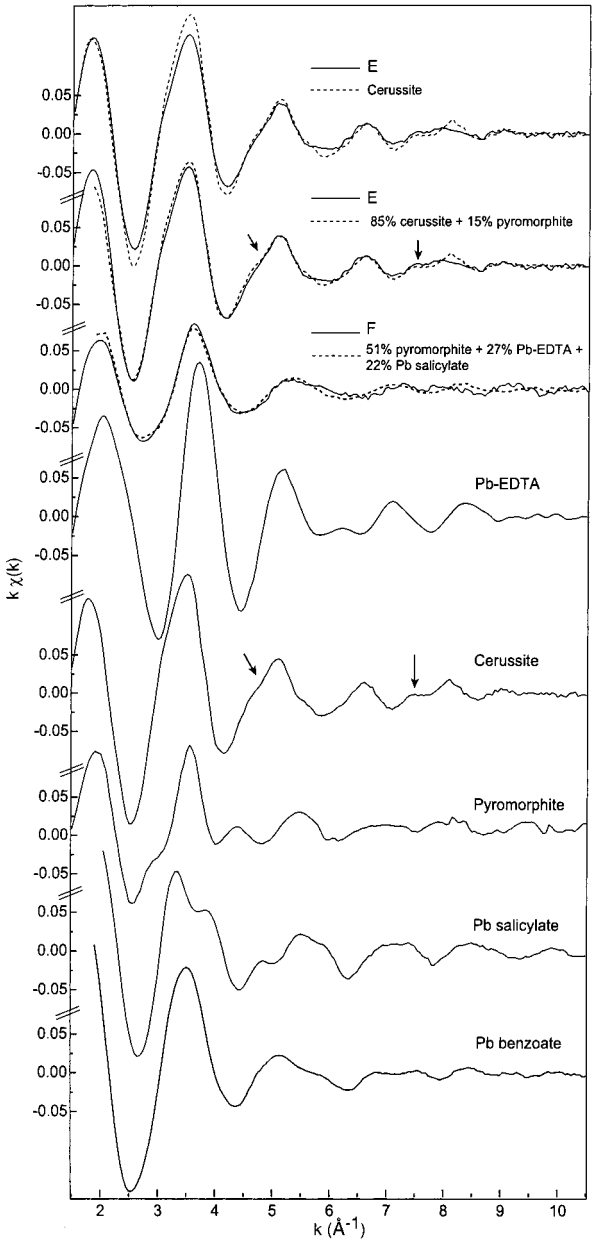

FIGURE 5. Comparison of Pb L_{III}-edge EXAFS spectra of the leaves for *P. vulgaris* grown in Pb nitrate- (E) and PbEDTA-containing nutrient solution (F), and for a selection of Pb model compounds. The sample "$PbEDTA^{2-}$" corresponds to a solution containing 0.01 mol/L $Pb(NO_3)_2$ and 0.04 mol/L Na_4EDTA at pH 4, whose calculated speciation is 100% $PbEDTA^{2-}$. The spectra were simulated by a linear combination of reference spectra (dotted lines)

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