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Calcium promotes cadmium elimination as vaterite grains by tobacco trichomes

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Abstract

In tobacco plants, elimination of Zn and Cd via the production of Ca-containing grains at the top of leaf hairs, called trichomes, is a potent detoxification mechanism. This study examines how Cd is incorporated in these biominerals, and how calcium growth supplement modifies their nature. Scanning electron microscopy coupled with energy dispersive X-ray microanalysis (SEM-EDX), microfocused X-ray diffraction (µ-XRD), and microfocused X-ray absorption near edge structure (µ-XANES) spectroscopy were used to image the morphology of the grains, identify the crystallized mineral phases, and speciate Cd, respectively. The mineralogy of the grains and chemical form of Cd varied with the amount of Ca. When tobacco plants were grown in a nutrient solution containing 25 µM Cd and low Ca supplement (Ca/Cd = 11 mol ratio), most of the grains were oblong-shaped and low-Cd-substituted calcite. When exposed to the same amount of Cd and high Ca supplement (Ca/Cd = 131 mol ratio), grains were more abundant and diverse in compositions, and in total more Cd was eliminated. Most grains in the high Ca/Cd experiment were round-shaped and composed predominantly of Cd-substituted vaterite, a usually metastable calcium carbonate polymorph, and subordinate calcite. Calcium oxalate and a Ca amorphous phase were detected occasionally in the two treatments, but were devoid of Cd. The biomineralization of cadmium and implications of results for Cd exposure of smokers and phytoremediation are discussed.

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1. INTRODUCTION

Plants have developed various defence strategies against toxic heavy metals, including complexation and chelation with strong ligands, and compartmentation into specific tissues, cells and cellular organelles (Clemens, 2006). Accumulation of metals inside trichomes, specialized cells located at the surface of leaves, is common and was documented, for example, in Brassica juncea L. (Salt et al., 1995), Alyssum lesbiacum (Krämer et al., 1997), Cucurbita moschata (Iwasaki and Matsumura, 1999), Nymphaea sp. (Lavid et al., 2001), and Arabidopsis halleri and thaliana (Küpper et al., 2000; Zhao et al., 2000; Sarret et al., 2002; 2009; Isaure et al., 2006). Heavy metals can also be excreted at the top of this hair-like appendage, but this process is less common and generally observed in halophyte species, such as Armeria maritima (Küpper et al., 2000; Sarret et al., 2006; 2007), and Atriplex halimus L. (Lefèvre et al., 2009). Tobacco (Nicotiana tabacum L. cv. Xanthi) detoxifies Zn and Cd by producing micrometer-sized Ca/ Zn and Ca/Cd-containing grains at the top of trichomes, similarly to halophytes (Choi et al., 2001; 2004; Choi and Harada, 2005; Sarret et al., 2006; 2007).

Biomineralization processes can be biologically induced or biologically controlled (Lowenstam, 1981). In the first case, the living organism modifies the physico-chemical conditions of its environment, so as to induce mineral precipitation near or at its surface. The organism has little control over the type and shape of minerals, which generally have heterogeneous morphology, composition, and structure. In biologically controlled biomineralization, nucleation, crystal growth, and the shape and size of crystallites can be controlled by biomolecules (Webb, 1999; Franceschi and Nakata, 2005).

The production of grain precipitated by tobacco plants is considered to be biologically induced, but the formation mechanism remains unclear (Sarret et al., 2006; 2007). The Ca/Zn grains produced under Zn and Zn + Ca exposures were 20–150 μm in diameter and polycrystalline aggregates of submicrometer crystals with some amorphous material. The crystals were composed dominantly of (Zn, Mg, Mn)-substituted calcite. Aragonite and vaterite, the two other CaCO₃ polymorphs, amorphous CaCO₃ and Ca oxalate (CaC₂O₄) monohydrate (whewellite) and dihydrate (weddellite) secondarily occurred, generally as an admixture of calcite. Other possible species included Zn complexed to organic compounds, Zn-containing silica and Zn phosphate. The proportion of Zn-substituted calcite relative to other Zn species and the density of trichomes increased with Ca, and in total more Zn was excreted.

As with Zn, trichomes produced 10–150 μm Ca/Cd grains when the plant roots were in contact with cadmium (Choi et al., 2001; 2004; Choi and Harada, 2005). Cd exposure retarded tobacco growth and doubled the density of trichomes per unit leaf area. Tolerance to metal toxicity was enhanced by adding Ca, which stimulated the production of grains (Choi et al., 2001). Because Cd and Ca form complete solid solutions in carbonates, as a result of their oxidation state and similar ionic radii (0.95 and 1.00 Å, respectively; Reeder, 1983), tolerance to Cd toxicity is probably linked to the production of calcium carbonate, but in a form and a manner as yet unknown. In this study, the nature of the Cd precipitates was investigated by growing tobacco plants in hydroponics in the presence of low and high Ca concentrations. The morphology, chemical composition, and crystalline nature of the Ca/Cd grains, and Cd speciation were characterized using scanning electron microscopy coupled with energy dispersive X-ray microanalysis (SEM-EDX), microfocused X-ray diffraction (µ-XRD), and microfocused Cd LIII-edge X-ray absorption near edge structure (µ-XANES) spectroscopy. The Cd species were identified by principal component analysis (PCA), and their proportions in grains quantified by linear least-squares combination fit (LSF) of the µ-XANES spectra.

2. MATERIALS AND METHODS

2.1. Plant cultures and isolation of grains from tobacco

Tobacco (Nicotiana tabacum L. cv. Xanthi) seeds were germinated on solid medium-filled PCR tubes, and transferred after three weeks to 1.5 L pots (three plants per pot) filled with one-tenth-strength Hoagland medium. To prevent insect attack and dust contamination, plants were grown at 22 °C in a closed culture box in a growth chamber with 16 h-light/8 h-dark cycle. After three weeks, plants were transferred to a medium containing 25 μM CdCl₂·2H₂O and 0.28 mM Ca (Cd treatment) or 3.28 mM Ca (Cd + Ca treatment). The treatment lasted 5 weeks. An experiment with 3.28 mM Ca only (Ca treatment) was performed for control, as described in Sarret et al. (2006). Grains were collected by plunging and vortexing plants in 50 mL tubes containing deionized water for a few seconds. The supernatant was carefully and quickly removed, and the grains at the bottom were collected with a pipette and dried in vacuum (Speed Vac SC100, Savant Instruments). The grains dissolve within 2 min at pH 2, within 10 min at pH 3 and are sparingly soluble from pH 4 to 12.5 (Sarret et al., 2006). Thus, water extraction did not modify the initial grain’s structure and composition.

2.2. Cd references

Solid and aqueous Cd-containing standards were prepared and analyzed by Cd LIII-edge XANES spectroscopy. Synthesis of Cd-phosphate (Cd₃(H₂PO₄)₄·4H₂O) and Cd-oxalate (CdC₂O₄), and the preparation of Cd₂O₃ and Cd organic compounds, including Cd-pectin, Cd-citr ate, Cd-malate, Cd-cell wall (Cd adsorbed on cell walls extracted from tobacco roots), and Cd-cysteine, were described previously (Isaure et al., 2006). Commercial powders of CdS, CdSO₄, CdCl₂, and CdCO₃ (stabilized) were purchased from Sigma–Aldrich, and their purity and crystallinity verified by XRD. In addition, Cd-containing calcite and vaterite were synthesized at room temperature by a protocol modified from Paquette and Reeder (1995) and Reeder (1996). Solid ammonium carbonate was introduced into a 50-mL Falcon tube floating in a sealed glass reactor containing 500 mL of 10 mM CaCl₂ and 1.8 M NH₄Cl. The second salt was used as a background electrolyte to provide a high
ionic strength. Initial pH was 4.9. The gradual decomposition of ammonium carbonate produced NH₃(g) and CO₂(g), which dissolved into the solution, increasing pH and alkalinity. The supersaturation of the unstrirred solution led to the nucleation and growth of CaCO₃ crystals. Continuous sublimation of NH₃(g) buffered the solution near pH 7.9.

After 13 days, the reactor contained rhombohedral crystals of calcite and spherical particles of vaterite attached to the surface of the glass. At this time, the CaCl₂–NH₄Cl solution was spiked slowly for 7 days with 0.1 M CdCl₂ to a total concentration of 100 µM Cd or 10 µM Cd. During this period, crystals continued to grow and Cd was incorporated as a Ca substituent in vaterite (Cd100-vaterite and Cd10-vaterite) and calcite (Cd100-calcite and Cd10-calcite). The gradual addition of CdCl₂ maintained the solution undersaturated with respect to otavite. Because Mg occurs in all grains produced by tobacco (Choi et al., 2001; 2004), (Cd, Mg)-substituted calcite ((Cd100, Mg100)-calcite) and (Cd, Mg)-substituted vaterite ((Cd100, Mg100)-vaterite) also were synthesized by co-adding 100 µM CdCl₂ and 100 µM MgCl₂ to a CaCl₂–NH₄Cl solution after 13 days and for 7 days. After 20 days, the particles from the three experiments were collected, rinsed with deionized water, and handpicked on the basis of their morphology. The distribution in size (150–200 µm) was independent of the morphology. SEM-EDX, XRD and microfocused X-ray fluorescence (µ-XRF) analyses showed that the rhombohedral crystals were pure Cd-containing or (Cd, Mg)-containing vaterite. Several tens of calcite and vaterite grains from the 100 µM Cd experiments were digested at 200 °C with pure HCl in Teflon bombs, and Cd concentrations analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES). The Cd10-calcite and Cd10-vaterite crystals could not be analyzed by ICP-AES due to the limited supply of material.

Cd-sorbed calcite was prepared following the protocol described in Elzinga and Reeder (2002). Briefly, 0.1 g of powdered calcite (Fluka) was controlled by XRD and equilibrated at ambient pressure and temperature in 1 L ultrapure water for 1 month. The suspension stabilized at pH 8.2 was spiked with 2 µmol Cd from a 0.01 M CdCl₂ solution. The pH remained constant for 2 days of equilibration, at which time the suspension was filtered, and the solid rinsed and dried for XANES measurements.

2.3. SEM-EDX analyses

Grains from tobacco and references were stuck on carbon stubs with carbon or kapton tape, coated with carbon, and examined with a Jeol-JSM 840A scanning electron microscope running at 20 keV and equipped with a Kevex Si(Li) diode EDX system. The chamber pressure was 10⁻⁶ to 10⁻³ Torr. Elemental concentrations were obtained by applying ZAF corrections (IDFix software).

2.4. µ-XRF, µ-XRD and µ-XANES data collection

The µ-XRF, µ-XRD, and some of the µ-XANES measurements were performed on beamline 10.3.2 at the Advanced Light Source (ALS) of the Lawrence Berkeley National Laboratory (Marcus et al., 2004). The tobacco grains were mounted on kapton tape (DuPont) and cooled to 150 K at ambient pressure with an Oxford Cryostream 611 cooler to minimize any potential beam damage during measurements. Cd-rich spots were localized by µ-XRF at an incident X-ray energy of 3550 eV (i.e., below Ca K-edge), and their µ-XRD patterns recorded with a 1024 × 1024 pixels Bruker Smart 6000 CCD camera at an incident energy of 17 keV and a beam size of 16 (H) × 7 (V) µm². Cd L₃-edge µ-XANES spectra were collected in fluorescence-yield detection mode on Cd-richest spots with a Canberra seven-element germanium detector and a beam size of 7 × 7 µm². Because Cd and Ar (from air) fluorescence emission lines overlap, µ-XRF and Cd L₃-edge µ-XANES measurements of the low-Cd samples were carried out in vacuum (10⁻⁷–10⁻⁸ Torr) on the spectromicroscopy beamline ID-21 at the European Synchrotron Radiation Facility (ESRF, Grenoble) using a one-element solid-state high purity germanium detector (Princeton Gamma Tech, Princeton, USA). Measurements on ID-21 were performed at room temperature and a beam size of 0.70 (H) × 0.35 (V) µm². The Cd L₃-edge µ-XANES spectra are averages of 3–20 successive scans collected on different spots.

2.5. µ-XRF and µ-XANES data analysis

The two-dimensional XRD patterns were calibrated with alumina (Al₂O₃) and integrated to one-dimensional patterns using fit2D software (Hammersley et al., 1996). The stoichiometry of the Mg-, Mn-, Zn-, and Cd-substituents in calcite crystals was estimated by refining the unit cell parameters a and c over the 10–33° 2θ angular range (1.3–4.0 Å interval at 17 keV) using the Ufit software (Evain, 1992), and applying the Vegard law (West, 1984). The end-members for Vegard law calculations were calcite, magnesite (MgCO₃), rhodochrosite (MnCO₃), smithsonite (ZnCO₃), and otavite (CdCO₃). The unit cell parameters a and c of the substituted vaterite crystals were refined but the stoichiometry of the substituents could not be estimated, because of the lack of metal carbonates isomorphic to vaterite.

The µ-XANES spectra were calibrated using the first inflection point of Cd metal set at 3538 eV, then pre-edge background subtracted with a linear polynomial and post-edge normalized with a linear or quadratic polynomial using the Athena software (Ravel and Newville, 2005). The normalized spectra were analyzed by principal components analysis (PCA; Ressler et al., 2000) using the beamline 10.3.2 LabView based software (Manceau et al., 2002). This numerical linear algebra analysis allows estimating the number of species required to describe the dataset, provided the number of species is smaller than the number of spectra and their fractional amounts vary in the dataset, and identifying their nature from a library of model compounds by target transformation. The number of principal components (i.e. Cd species) was evaluated with the IND local minimum criterion, and the quality of the reconstruction of the reference spectra by
target transformation with the \textit{SPOIL} value (S) and the normalized sum-squared residual \textit{NSS} = \left[ \frac{\sum (\text{Normalized Absorption}_{\text{th}} - \text{Normalized Absorption}_{\text{exp}})^2}{\sum (\text{Normalized Absorption}_{\text{th}})^2} \right] \times 100 \text{ in the 3530–3585 eV range (Malinowski, 1977, 1978; Manceau et al., 2002).}

Then, the proportions of Cd species in the multi-component XANES spectra were obtained by least-squares fitting (LSF) of the grain spectra to linear combinations of reference spectra previously identified by PCA. The quality of the fits was quantified with \textit{NSS}. Visual examination of all individual fits showed that the grains contained at most two major species; the addition of a second component being justified when \textit{NSS} decreased by at least 40\%. Spectra were checked for possible over-absorption as described in Sarret et al. (2007) using the beamline 10.3.2 LSF LabView based software, and this effect was not observed.

3. RESULTS

3.1. Morphology and chemical composition of the grains

3.1.1. Abiotic grains

The vaterite grains were all spherical and the calcite grains rhombohedral (Fig. 1a and b). The concentration of Cd in Cd100-calcite was heterogeneous within each grain and between grains, with Cd contents varying from 1 to 60 mg g\(^{-1}\) Cd, as estimated by EDX. Cadmium was below...
the detection limit of EDX in Cd100-vaterite, but was detected by μ-XRF. The average Cd contents obtained by ICP-AES were 1.8 mg g\(^{-1}\) for Cd100-calcite and 54 μg g\(^{-1}\) for Cd100-vaterite. From μ-XRF analysis, Cd10-vaterite contained less Cd than Cd10-calcite, and (Cd100, Mg100)-vaterite less than (Cd100, Mg100)-calcite. Magnesium decreased the amount of Cd incorporated in both calcite and vaterite.

### 3.1.2. Cd + Ca treatment

About twenty grains were examined. Their size ranged from 20 to 150 μm, and approximately 75% were rounded to sub-rounded and composed of minute (<1 μm) particles, suggesting the predominance of vaterite (Fig. 1c and d). From EDX analysis, they all contained Ca, Mg, and Cd. The Cd concentration varied from 9 to 55 mg g\(^{-1}\) with a mean value of approximately 29 mg g\(^{-1}\). About 20% of the grains contained faceted calcite-like crystals. Some did not contain Cd in contrast to the rounded grains, and the mean concentration of these faceted Cd grains was 17 mg g\(^{-1}\) (Fig. 1e and f). Thus, Cd seems to have a higher affinity for biogenic vaterite than for calcite. The reverse trend was observed for the inorganic Cd100-calcite and Cd100-vaterite references: Cd was more abundant in calcite than in vaterite grains. Minor Al, S, Cl, and K were detected regardless of morphologies. The other grains appeared amorphous; Ca was still the major element present, but Cd was never detected (results not shown). An intense Si K\(\alpha\) peak was observed when grains were mounted on Kapton, but not on carbon tape (e.g., grains CdCaG8, Fig. 1f). This peak is likely an artifact coming from a secondary excitation of Kapton by the Ca K\(\alpha\) fluorescence emitted by the grains.

### 3.1.3. Cd treatment

The grain size varied between 20 and 150 μm and the grain shape oblong, except for three grains out of approximately 20 grains examined, which were rounded. The oblong grains were composed of micrometer (Fig. 1g) to submicrometer (Fig. 1h and i) crystals and had no detectable Cd. Neither calcite nor vaterite could be recognized from the grain or crystal morphologies. One hemispherical grain was analyzed by EDX (CdCaG12), and found to contain Cd (Fig. 1j).

### 3.1.4. Ca treatment

Most grains were composed of faceted calcite-like crystals, and contained Mg and occasionally Mn impurities, as described in Sarret et al. (2006).

### 3.2. Mineralogy of the grains

#### 3.2.1. Cd + Ca treatment

Twelve grains were examined by μ-XRD (Table 1). CdCaG1 is representative of the most frequently observed rounded grains (CdCaG1 to CdCaG7). The intensities of the Debye rings are not constant along the ring perimeters, and are characteristic of submicrometer crystals with preferred orientations (Fig. 2a) (Manca et al., 2002). The texture looks like several lumps of mosaic crystals squashed together, as further discussed below regarding the stability of calcium carbonates. The XRD peaks are from vaterite only, but shifted to higher wavevector \(Q\) values (i.e. smaller \(d\) values) relative to pure vaterite (Fig. 2c). The contraction of the unit cell from \(a = 7.148\) Å and \(c = 16.949\) Å in vaterite to \(a = 7.06 \pm 0.01\) Å and \(c = 16.72 \pm 0.01\) Å in CdCaG1 likely results from the substitution of Mg (ionic radius

![Fig. 1 (continued)](image-url)
\( r = 0.72 \) Å, Mn \( (r = 0.83 \) Å), and Cd \( (r = 0.95 \) Å) for Ca \( (r = 1.00 \) Å; Shannon, 1976), based on EDX and \( \mu \)-XRF analyses (Figs. 1d and 2d). The other rounded grains were composed also of substituted minute vaterite crystals. Their average composition varied from grain to grain, with \( a \) ranging from 7.06 Å to 7.09 Å and \( c \) from 16.70 Å to 16.81 Å, but likely also between crystallites within the same grain.

CdCaG8 and CdCaG9 have only faceted crystals. Their spotty \( \mu \)-XRD patterns (Fig. 2b) indicate that only a limited number of crystals were in Bragg condition in the \( \sim 16 \times 7 \times 10 \) μm\(^2\) diffraction volume (horizontal \times vertical size of the beam \times estimated thickness of the grain). Thus, the crystals sizes are relatively coarse. The unit cell parameters are again smaller (\( a = 4.91 \) Å and 4.92 ± 0.01 Å, \( c = 16.79 \) and 16.83 ± 0.01 Å) than those of the pure CaCO\(_3\) polymorph, here calcite (\( a = 4.9896 \) Å, \( c = 17.0610 \) Å). EDX and \( \mu \)-XRF showed that Mg and Cd, and to a lesser extent Mn and Zn, are the most likely Ca substituents (Figs. 1f and 2e).

### Table 1

<table>
<thead>
<tr>
<th>Carbonate minerals</th>
<th>Unit cell parameters</th>
<th>Ionic radius for divalent cations ( r ) (Å)</th>
<th>References (Shannon, 1976)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite (CaCO(_3))</td>
<td>4.986 17.0610</td>
<td>1.00</td>
<td>Effenberger et al. (1981)</td>
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<tr>
<td>Otavite (CdCO(_3))</td>
<td>4.923 16.287</td>
<td>0.95</td>
<td>Borodin et al. (1979)</td>
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<td>Rhodocrosite (MnCO(_3))</td>
<td>4.7682 15.6354</td>
<td>0.83</td>
<td>Effenberger et al. (1981)</td>
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<td>Smithsonite (ZnCO(_3))</td>
<td>4.6526 15.0257</td>
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<td>Magnesite(MgCO(_3))</td>
<td>4.6328 15.0129</td>
<td>–</td>
<td>–</td>
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<td>Vaterite (CaCO(_3))</td>
<td>7.148 16.949</td>
<td>0.72</td>
<td>Meyer (1969)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grains</th>
<th>Unit cell parameters</th>
<th>Mineralogy</th>
<th>Average amounts of metal substituents in calcite calculated from the ( a ) parameter</th>
<th>Average amounts of metal substituents in calcite calculated from the ( c ) parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>CdCaG1</td>
<td>7.06 16.72</td>
<td>Substituted vaterite</td>
<td>0.22 0.24 0.36 1.00 (^a)</td>
<td>0.13 0.13 0.19 0.35</td>
</tr>
<tr>
<td>CdCaG2</td>
<td>7.07 16.72</td>
<td>Substituted vaterite</td>
<td>0.19 0.21 0.31 1.00 (^a)</td>
<td>0.11 0.11 0.16 0.30</td>
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<tr>
<td>CdCaG3</td>
<td>7.08 16.78</td>
<td>Substituted vaterite</td>
<td>0.17 0.18 0.27 0.89</td>
<td>0.17 0.18 0.24 0.44</td>
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<tr>
<td>CdCaG4</td>
<td>7.07 16.79</td>
<td>Substituted vaterite + W(t)</td>
<td>0.22 0.24 0.40 1.00 (^a)</td>
<td>0.26 0.26 0.38 0.70</td>
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<tr>
<td>CdCaG5</td>
<td>7.09 16.81</td>
<td>Substituted vaterite + W(t)</td>
<td>0.22 0.24 0.36 1.00 (^a)</td>
<td>0.25 0.25 0.36 0.66</td>
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<tr>
<td>CdCaG6</td>
<td>7.08 16.79</td>
<td>Substituted vaterite + substituted calcite (t) + W(t)</td>
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<td>0.11 0.11 0.16 0.30</td>
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<tr>
<td>CdCaG7</td>
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<td>Substituted vaterite + substituted calcite (t)</td>
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<tr>
<td>CdCaG8</td>
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<td>Substituted calcite</td>
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<td>0.13 0.13 0.19 0.35</td>
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<tr>
<td>CdCaG9</td>
<td>4.92 16.83</td>
<td>Substituted calcite</td>
<td>0.19 0.21 0.31 1.00 (^a)</td>
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<td>CdCaG10</td>
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<td>Substituted calcite + substituted vaterite</td>
<td>0.17 0.18 0.27 0.89</td>
<td>0.17 0.18 0.24 0.44</td>
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<tr>
<td>CdCaG11</td>
<td>4.93 16.89</td>
<td>Substituted calcite + substituted vaterite</td>
<td>0.17 0.18 0.27 0.89</td>
<td>0.09 0.09 0.13 0.23</td>
</tr>
<tr>
<td>CdCaG12</td>
<td>7.09 16.69</td>
<td>No ( \mu )-XRD peaks</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Grains from the Cd + Ca treatment</th>
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<tbody>
<tr>
<td>CdG1</td>
</tr>
<tr>
<td>CdG2</td>
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<td>CdG3</td>
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<tr>
<td>CdG4</td>
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<td>CdG15</td>
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<td>CdG16</td>
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</tbody>
</table>

\(^a\) Value set to 1 because the \( a \) parameter is equal or smaller than the value for the CdCO\(_3\) end-member (otavite). W, whewellite (CaC\(_2\)O\(_4\)/C\(_1\)H\(_2\)O); A, aragonite (CaCO\(_3\)); (t), trace amounts. –, element not detected by \( \mu \)-XRF and EDX.
Fig. 2. Two-dimensional (a and b) and one-dimensional μ-XRD patterns (c), and μ-XRF spectra (d and e) for the rounded grain CdCaG1 and the faceted grain CdCaG8, both from the Cd + Ca treatment. Grain CdCaG1 contains nanometer to submicrometer-sized vaterite crystals with preferential orientation, and grain CdCaG8 nanometer to coarse calcite crystals. Diffraction peaks are shifted to shorter $d$ values (higher $Q$ values) as a result of Cd, Mn, Zn, and/or Mg for Ca substitutions. The (1 1 0) reflection of the CdCaG8 grain (inset) is at $d = 2.455 \, \text{Å}$, compared to $2.495 \, \text{Å}$ for calcite, $2.461 \, \text{Å}$ for otavite (CdCO$_3$), $2.384 \, \text{Å}$ for rhodocrosite (MnCO$_3$), $2.326 \, \text{Å}$ for smithsonite (ZnCO$_3$), and $2.316 \, \text{Å}$ for magnesite (MgCO$_3$).
Assuming that each diffracting calcite crystal contains only one substitutional cation, a theoretical stoichiometry can be calculated from the Vegard law using the $a$ or $c$ parameters (Fig. 3). The precision with $c$ is higher, because this metrical parameter is more sensitive to the substitution rate than is $a$. For an uncertainty on $a$ and $c$ of ±0.01 Å, the precisions in mole fractions are ±1.3% of total cation for Cd, 0.7% for Mn, 0.5% for Zn, and 0.5% for Mg with $c$, and ±15% Cd, 4.5% Mn, 3.0% Zn, and 2.8% Mg with $a$. Using $c$, the calcite crystals in CdCaG8 contain on average and at most, 13–14 at.% Mg, 13–14 at.% Zn, 18–20 at.% Mn, and 34–36 at.% Cd. The $a$ parameter gives 19–25 at.% Mg, 20–27 at.% Zn, and 31–40 at.% Mn. CdCaG9 has the same average degree of substitution as CdCaG8 (Table 1), despite the generally heterogeneous composition of individual crystallites in trichome grains (Sarret et al., 2007). The maximum mole fraction of Cd could not be calculated in the two grains, because the experimental $a$ value (4.91 and 4.92 ± 0.01 Å) is smaller or identical to that of the pure end-member otavite (4.923 Å). Therefore, the contraction of the unit cell is not due to Cd alone, this impurity coexists with at least one smaller cation, likely Mg because this was the main impurity in calcite from the Ca treatment.

Within precision, the level of metal substitution seems to be higher in the $a$ than in the $c$ direction. This anisotropic lattice distortion may have two origins, which both result in a lesser reduction of the $c$ parameter relative to $a$. One is the disordering of cations over the two nonequivalent Ca sites of calcite, as in thermally-treated dolomite (CaMg(CO$_3$)$_2$; Reeder and Wenk, 1983; Bromiley et al., 2007). The other is the incorporation in the structure of organic molecules, as in biogenic carbonates from mollusk shells (Pokroy et al., 2006; Zolotoyabko and Pokroy, 2007).

The other grains (CdCaG10 and CdCaG11) contain calcite and vaterite. Vaterite has about 2.5 times more Cd than calcite. The Ca oxalate monohydrate whewellite (W, Ca$_2$C$_2$O$_4$·H$_2$O) occurred occasionally as trace mineral in some grains. One grain (CdCaG12) did not diffract nor had detectable Cd, although it was rich in Ca, probably in an amorphous phase.

3.2.2. Cd treatment

Sixteen grains were examined by μ-XRD (Table 1). Calcite grains were most prevalent and generally more substituted than in the Ca + Cd treatment. Their μ-XRD patterns showed spots from relatively coarse calcite crystals mixed with continuous Debye rings from minute calcite particles (CdG1, CdG3, Fig. 4). CdG12 was the only grain with substituted vaterite in addition to substituted calcite (Fig. 5). When present, whewellite and aragonite were subordinate to calcite and vaterite. Four Ca-rich grains did not produce a diffraction signal, which suggests a non-crystalline Ca-containing organic or inorganic phase. Cadmium was not detected, except for CdG12.
3.2.3. Ca treatment

The grains consisted mainly of (Mg, Mn)-substituted calcite, along with subordinate amounts of whewellite (CaC_2O_4·H_2O), weddellite (CaC_2O_4·2H_2O), and vaterite, as previously reported for this type of treatment (Sarret et al., 2006, 2007).

3.3. Cd speciation

3.3.1. Reference compounds

The Cd L_{III}-edge μ-XANES spectra of the references have a peak at 3540 eV when Cd is bonded to O ligands, not when it is bonded to S ligands, as observed for CdS and Cd-cysteine (Fig. 6; Pickering et al., 1999; Isuere et al., 2006). Among the references with O ligands, those in which Cd is bound to COOH/OH groups (Cd-cell wall, Cd-malate, Cd-citrate, Cd-oxalate and Cd-pectin) and to phosphate (Cd-phosphate) are featureless compared to the other references and to aqueous Cd (Cd_{aq}). The six Cd-containing calcium carbonate spectra are well structured, and distinct from the other reference spectra. The spectra of otavite and Cd-containing calcites show a pronounced peak at 3551 eV, but the otavite spectrum has in addition a shoulder at 3547 eV (arrow in Fig. 6). The similarity of the spectra for the four Cd-containing calcites (Cd10, Cd100, (Cd100, Mg100), and Cd-sorbed) suggests a common structural geometry of their bonding environment. This observation is consistent with the formation of a (Ca,Cd)CO_3 solid solution at the calcite surface exposed to 0.1 μM Cd for 24 h (Chada et al., 2005). In the following, the four samples are named by the same generic expression “Cd-containing calcite”. A lower amplitude of the peak at 3551 eV is distinctive of Cd100-vaterite.

3.3.2. Nature of Cd species in tobacco grains

All tobacco grain spectra show a peak at 3540 eV, indicating that Cd is coordinated to oxygen atoms (Fig. 6). Six
out of the nine Cd + Ca grains spectra resemble Cd100-vaterite, whereas the others (CdCaG7, CdCaG8, and CdCaG9) have more similarities with Cd-containing calcite. Only two spectra could be recorded for the Cd treatment (CdG1 and CdG12), because of the low concentration of Cd. Although extremely noisy, the CdG1 spectrum resembles Cd-containing calcite, whereas CdG12 has more similarities with Cd100-vaterite.

PCA was performed on the whole set of tobacco grain spectra, except CdG1. Based on the IND local minimum criterion, the dataset contains three independent components (i.e., Cd species; Fig. 7). However, examination of the first five principal components suggests that only the first two may be meaningful; the third is weak and does not appear sufficiently modulated to justify its inclusion in the PCA. Because the IND determination method is not fully accepted (Manceau et al., 2002; Sarret et al., 2004; Panfili et al., 2005; Kirpichtchikova et al., 2006; Manceau and Matynia, 2010), and visual inspection is too subjective, the target transformations were performed with two and three components.

Good spectral match of the reference spectra to the principal components were obtained with Cd-containing calcite ($NSS = 4.2 \times 10^{-4}$, $S = 0.7$, and $NSS = 3.8 \times 10^{-4}$, $S = 1.8$, with two and three principal components, respectively) and Cd100-vaterite ($NSS = 7.6 \times 10^{-4}$, $S = 3.0$ and $NSS = 2.8 \times 10^{-4}$, $S = 2.3$; Fig. 8). The spectra for Cd-cell wall ($NSS = 17.2 \times 10^{-4}$, $S = 3.0$ and $NSS = 14.3 \times 10^{-4}$, $S = 4.7$), Cd-pectin ($NSS = 32.1 \times 10^{-4}$, $S = 3.6$, and $NSS = 24.6 \times 10^{-4}$, $S = 4.7$, not shown), Cd-cellulose ($NSS =
19.2 \times 10^{-4}, S = 3.9, and NSS = 18.3 \times 10^{-4}, S = 6.8, not shown), and Cd-malate (NSS = 21.2 \times 10^{-4}, S = 5.0, and NSS = 19.9 \times 10^{-4}, S = 8.1) considered as proxies for Cd bound to COOH/OH groups of organic compounds, and
Cd-phosphate (NSS = 4.11, S = 5.5, and NSS = 35.2, S = 7.2), otavite (NSS = 21.6, S = 3.6, and NSS = 13.0, S = 6.9) were not well reconstructed, regardless of the number of principal components used in the target transformation. These results suggest that Cd is mainly precipitated as carbonates in all grains.

3.3.3. Proportions of Cd species in tobacco grains

The proportions of the two Cd carbonate species (i.e., calcite and vaterite) in the nine grains from the Cd + Ca treatment and the two grains from the Cd treatment (CdG1, CdG12) were determined next by LSF of the experimental spectra to linear combinations of the Cd-containing calcite and Cd100-vaterite reference spectra (Table 2, Fig. 9). Best one-component fits were obtained with Cd-containing calcite for CdCaG7, CdCaG8, CdCaG9, and CdG1, and with Cd100-vaterite for the other grains. Adding Cd100-vaterite to the calcitic grains much improved the reconstruction of CdCaG7 (NSS decreased from 20 \times 10^{-4} to 3.7 \times 10^{-4}), but not those of the three other grains. However, the NSS values of CdCaG8 and CdCaG9 could be reduced by 40% upon adding 16–18% of a Cd-organic species. This species is minor (close to the detection limit), and there was no other circumstantial evidence for its presence in other grains, which may explain why it was not identified by PCA. Organically bound Zn and Ca also were minor species in the tobacco grains studied previously (Sarret et al., 2007). The two-component fits of the grain spectra, in which Cd100-vaterite was dominant, identified Cd-containing calcite in two grains: CdCaG4 and CdG12.

Thus, among the eleven grains examined by XANES, Cd is present only or predominantly in calcite in three grains (CdCaG8, CdCaG9 and CdG1), evenly distributed between calcite and vaterite in one grain (CdCaG7), and only or predominantly in vaterite in the others. Four grains (CdCaG1, CdCaG7, CdCaG12, CdG12) were studied previously by Ca-XANES (Sarret et al., 2007). Calcite and vaterite had been also identified by this technique, except CdCaG12 in which the Ca carbonate species was non-crystalline (Table 1).

3.4. Relationship between Cd speciation and morphology/mineralogy of the tobacco grains

The nature of the Cd host phases, as determined by \( \mu \)-XANES, is consistent with the mineralogy of the crystalline
phases, as determined by μ-XRD, except for CdCaG4 (Table 3). Vaterite was identified by the two techniques in this grain, but the second species, Cd-substituted calcite, only by spectroscopy. A likely reason is the difference in sample volume probed by the two techniques. For μ-XRD measurements, the beam size was 16 × 7 μm² and the penetration depth from several tens to a few hundreds of μm at 17 keV, whereas they were 0.70 × 0.35 μm² and a few μm at the Cd LIII-edge for μ-XANES. Thus, the detection by μ-XANES of calcite in this vaterite-rich grain suggests that it contains euhedral calcite crystals at its surface, as observed for CdG12 (Fig. 1j).

4. DISCUSSION

4.1. Calcium biomineralization and Cd elimination in tobacco grains

Calcium carbonate is a common biogenic mineral in the Animalia kingdom (Weiner and Dove, 2003). In Plantae, it is biosynthesized in few families, such as Moraceae, Urticaceae, and Acanthaceae, generally occurring as intercellular concretions (cystolith) (Arnott and Pautard, 1970; Setoguchi et al., 1989; Nitta et al., 2006). In contrast to calcium carbonate in animals, calcium oxalate is probably the most commonly formed mineral in higher plants (Franceschi and Homer, 1980). The monohydrated form (whewellite) is more frequent than the anhydrous (weddelite). These crystals can form in most tissues and organs, such as roots, bark, stems, leaves, flowers, fruits, and seeds (Arnott and Pautard, 1970). In tobacco, they occur in the vacuoles of specialized cells called idioblasts (Bouropoulos et al., 2001). Oxalate biocrystals play a central role in a variety of important functions, including tissue calcium regulation, osmotic balance, protection from herbivory, metal detoxification, and improvement of the mechanical properties of the tissues (Nakata, 2003; Franceschi and Nakata, 2005). Intra-cellular detoxification of Cd by calcium oxalate was reported in water hyacinth (Mazen and El Maghraby, 1997).

In tobacco, calcium has a protective effect against Cd toxicity: the amounts of grains per mass of dry matter is low under Cd exposure alone, moderate under Ca exposure, and high under Cd + Ca exposure (Choi et al., 2001; Choi and Harada, 2005). Apparently, calcium does not compete with Cd for uptake by roots, but instead pro-

Table 2
Proportion of Cd species (in% mole fraction) determined by LSF of the Cd L III-edge μ-XANES spectra.

<table>
<thead>
<tr>
<th>Grains from the Cd + Ca treatment</th>
<th>Cd-containing calcite (%)</th>
<th>Cd100-vaterite (%)</th>
<th>Cd-organic (%)</th>
<th>Sum</th>
<th>NSSbest* (× 10³)</th>
<th>P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CdCaG1</td>
<td>One component</td>
<td>85</td>
<td>85</td>
<td>5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CdCaG3</td>
<td>One component</td>
<td>93</td>
<td>93</td>
<td>3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CdCaG4</td>
<td>One component</td>
<td>96</td>
<td>96</td>
<td>8.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two components</td>
<td>30</td>
<td>67</td>
<td>97</td>
<td>3.0</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>CdCaG6</td>
<td>One component</td>
<td>91</td>
<td>91</td>
<td>3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CdCaG5</td>
<td>One component</td>
<td>95</td>
<td>95</td>
<td>4.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CdCaG7</td>
<td>One component</td>
<td>104</td>
<td>104</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two components</td>
<td>52</td>
<td>51</td>
<td>103</td>
<td>3.7</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>CdCaG8</td>
<td>One component</td>
<td>90</td>
<td>90</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two components</td>
<td>76</td>
<td>16</td>
<td>92</td>
<td>3.7</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>CdCaG9</td>
<td>One component</td>
<td>98</td>
<td>98</td>
<td>6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two components</td>
<td>83</td>
<td>18</td>
<td>101</td>
<td>3.5</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>CdCaG13</td>
<td>One component</td>
<td>87</td>
<td>87</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grains from the Cd treatment</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CdG1</td>
<td>One component</td>
<td>107</td>
<td>107</td>
<td>311</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CdG12</td>
<td>One component</td>
<td>105</td>
<td>105</td>
<td>3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two components</td>
<td>15</td>
<td>91</td>
<td>106</td>
<td>2.2</td>
<td>39</td>
<td></td>
</tr>
</tbody>
</table>

a Residual (normalized sum squares) between fit and experimental data: \( NSS_{best} = \left[ \frac{\sum (\text{Normalized Absorption}_{\text{th}} - \text{Normalized Absorption}_{\text{exp}})^2}{\sum \text{Normalized Absorption}_{\text{exp}}} \right] \times 100 \), in the 3530–3585 eV interval.

b Improvement of the fit: \( I = \frac{(NSS_{n \text{ components}} - NSS_{n + 1 \text{ component}}/NSS_{n \text{ components}}) \times 100}{NSS_{n \text{ components}}} \). The fit components are precise to 20% of total Cd. The precision on Cd-organic is slightly better, approximately 10–15%. The sum of the Cd component(s) deviates from 100% for two reasons, one is the precision of each component, the other is the accuracy of the method itself. See Van Damme et al. (2010) for details.
vides powerful synergy for Cd detoxification by trichomes. Calcium does more than increasing the density of trichomes (<20 μm; Choi et al., 2001) lead to the conclusion that precipitation results from the contact with atmosphere of a Ca-rich exudate. The heterogeneity and variability in morphology, composition and structure of the grains also are suggestive of a “biologically induced” process (Sarret et al., 2006). The general relationships between the shape (spherical vs. faceted) and mineralogy (vaterite vs. calcite) of the grains, on one hand, and the amount of Ca in the nutritive solution (Cd + Ca vs. Cd treatment), on the other, reinforces the idea that this biomineralization does not depend on the plant cellular activity itself but is determined by the chemistry of exudates.

This difference may result from the presence of organics in biotic grains.

4.2. Biologically induced or biologically controlled mechanism?

The mechanism of grain formation is as yet unknown. Considerations of the size of the grains (20–150 μm) and diameter of the head of trichomes (<20 μm; Choi et al., 2001) lead to the conclusion that precipitation results from the contact with atmosphere of a Ca-rich exudate. The heterogeneity and variability in morphology, composition and structure of the grains also are suggestive of a “biologically induced” process (Sarret et al., 2006). The general relationships between the shape (spherical vs. faceted) and mineralogy (vaterite vs. calcite) of the grains, on one hand, and the amount of Ca in the nutritive solution (Cd + Ca vs. Cd treatment), on the other, reinforces the idea that this biomineralization does not depend on the plant cellular activity itself but is determined by the chemistry of exudates.

4.3. Occurrence and stabilization of vaterite

Vaterite is thermodynamically the most unstable polymorph of the three crystal structures of CaCO₃, and rapidly transforms into aragonite or calcite (Ogino et al., 1987; Kralj et al., 1997; Vecht and Ireland, 2000; Nehrke and Van Cappellen, 2006; Rieger et al., 2007). It can be stabilized by organic molecules (Collen and Antonietti, 1998; Naka and Chuo, 2001; Falini et al., 2005; Rodriguez-Navarro et al., 2007; Qiao et al., 2008), such as proteins (Kanakis and Dalas, 2000; Rautaray et al., 2005), exopolysaccharides and carboxylic molecules (Dalas et al., 1999; Manoli and Dalas, 2001; Braissant et al., 2003; Malkaj and Dalas, 2004; Naka et al., 2006), and amino acids (Manoli et al., 2002; Lakshminarayanan et al., 2005; Xie et al., 2005). Consequently, abiogenic vaterite is rare in nature (Rowlands and Webster, 1971; Lucas and Andrews, 1996), and more common in biota (Lowenstam and Weiner, 1989; Mann, 2001). Reported occurrences include the spicule of Ascidia cea marine organisms (Lowenstam and Abbott, 1975), fish otoliths (Cyprinids) (Falini et al., 2005; Lenaz et al., 2006), freshwater pearls (Qiao and Feng, 2007; Qiao et al., 2008), microbial mats from lake sediments (Giralt et al., 2001), avian and turtle eggshells (Dennis et al., 1996; Lakshminarayanan et al., 2005), larval shell of a freshwater snail (Hasse et al., 2000), and human gallstones where it is associated with cholesterol (Palchik and Moroz, 2005).

At the trichome surface, the biomineralization of vaterite may occur by a mechanism analogous to the protein-mediated reaction of aqueous Ca²⁺ with CO₂, and the subsequent precipitation of stable vaterite crystals of spherical morphology described at the surface of actinomycetes, fungi, and chickpea roots (Rautaray et al., 2004; 2005). Tobacco trichomes excrete organic substances, including alkaloids such as nicotine, terpenoids (Callow et al., 2000), and defensive proteins (Wagner et al., 2004; Shepherd et al., 2005; Schilmiller et al., 2008). Further investigations are needed to determine the exact nature of organic compounds in

Fig. 9. Cd LIII-edge μ-XANES spectra for tobacco grains (solid lines) and best linear combination fits (dashed lines) with one or two components (CdCaG4, CdCaG7, CdCaG8, CdCaG9, and CdG12).
contact with vaterite crystals, and their possible role in the
texturing of the vaterite microcrystals (Fig. 2a). Micro-
twinning of biogenic vaterite along the (0 0 1) direction was
observed by high-resolution transmission electron micros-
copy (HRTEM) and selected area electron diffraction
(SAED) in freshwater lackluster pearls (Qiao and Feng,
2007). The 
-XRD pattern from grain CdCaG1 suggests that
the vaterite crystals are a mosaic of crystalline blocks tilted to
each other by stacking faults, typically by fractions of a min-
ute of arc.

The transformation of vaterite into calcite may be poi-
soned also by substitutional impurities, such as Mn
2+ (Nassrallah-Aboukais et al., 1998a), Cu
2+ (Nassrallah-
Aboukais et al., 1998b) and Mg
2+ (Kitamura, 2001; Nishi-
no et al., 2009), but is hastened by Cd
2+ and Pb
2+. This
explanation apparently does not apply to biogenic carbon-
ates, because the calcite crystals produced by tobacco gen-
erally hold more trace metals than vaterite (Table 1), and
vaterite has more cadmium.

4.4. Health care and environmental implications

Tobacco smoking is one of the main exposure routes
for humans to heavy metals and particularly to Cd
(Lugon-Moulin et al., 2004; Stephens et al., 2005). Stim-

Table 3
Summary of the information obtained for each tobacco grain.

<table>
<thead>
<tr>
<th>Grains from the Cd + Ca treatment</th>
<th>Cd speciation (μ-XANES)</th>
<th>Ca speciation (μ-XANES) a</th>
<th>Mineralogy (μ-XRD)</th>
<th>Morphology (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CdCaG1</td>
<td>85% Cd-vaterite</td>
<td>80% vaterite + 20% organic Ca and/or ACC</td>
<td>Substituted vaterite</td>
<td>Rounded</td>
</tr>
<tr>
<td>CdCaG2</td>
<td>N.m.</td>
<td>N.m.</td>
<td>Substituted vaterite</td>
<td>Rounded</td>
</tr>
<tr>
<td>CdCaG3</td>
<td>93% Cd-vaterite</td>
<td>N.m.</td>
<td>Substituted vaterite + W(t)</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdCaG4</td>
<td>67% Cd-vaterite + 30% Cd-containing calcite</td>
<td>N.m.</td>
<td>Substituted vaterite + W(t)</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdCaG5</td>
<td>95% Cd-vaterite</td>
<td>N.m.</td>
<td>Substituted vaterite + W(t)</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdCaG6</td>
<td>91% Cd-vaterite</td>
<td>N.m.</td>
<td>Substituted vaterite + substituted calcite (t) + W(t)</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdCaG7</td>
<td>52% Cd-containing calcite + 48% Cd-vaterite</td>
<td>90% calcite + 10% organic Ca and/or ACC</td>
<td>Substituted vaterite + substituted calcite (t)</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdCaG8</td>
<td>76% Cd-containing calcite + 24% 16% Cd-organic</td>
<td>N.m.</td>
<td>Substituted calcite</td>
<td>Faceted</td>
</tr>
<tr>
<td>CdCaG9</td>
<td>83% Cd-containing calcite + 18% Cd-organic</td>
<td>N.m.</td>
<td>Substituted calcite</td>
<td>Faceted</td>
</tr>
<tr>
<td>CdCaG10</td>
<td>N.m.</td>
<td>N.m.</td>
<td>Substituted calcite + substituted vaterite</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdCaG11</td>
<td>N.m.</td>
<td>N.m.</td>
<td>Substituted calcite + substituted vaterite</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdCaG12</td>
<td>No Cd signal</td>
<td>70% ACC + 30% undetermined</td>
<td>No μ-XRD peak</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdCaG13</td>
<td>87% Cd-vaterite</td>
<td>N.m.</td>
<td>No l-XRD peak</td>
<td>N.o.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grains from the Cd treatment</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CdG1</td>
<td>107% Cd-containing calcite</td>
<td>N.m.</td>
<td>Substituted calcite</td>
<td>Oblong</td>
</tr>
<tr>
<td>CdG2</td>
<td>No Cd signal</td>
<td>N.m.</td>
<td>Substituted calcite + W(t)</td>
<td>Oblong</td>
</tr>
<tr>
<td>CdG3</td>
<td>No Cd signal</td>
<td>N.m.</td>
<td>Substituted calcite</td>
<td>Oblong</td>
</tr>
<tr>
<td>CdG4</td>
<td>No Cd signal</td>
<td>N.m.</td>
<td>Substituted calcite + A</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdG5</td>
<td>No Cd signal</td>
<td>N.m.</td>
<td>Substituted calcite</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdG6</td>
<td>No Cd signal</td>
<td>N.m.</td>
<td>Substituted calcite</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdG7</td>
<td>No Cd signal</td>
<td>N.m.</td>
<td>Substituted calcite + W + A(t)</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdG8</td>
<td>No Cd signal</td>
<td>N.m.</td>
<td>Substituted calcite + W(t)</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdG9</td>
<td>No Cd signal</td>
<td>N.m.</td>
<td>Substituted calcite</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdG10</td>
<td>N.m.</td>
<td>N.m.</td>
<td>Substituted calcite</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdG11</td>
<td>N.m.</td>
<td>N.m.</td>
<td>Substituted calcite + W(t)</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdG12</td>
<td>91% Cd-vaterite + 15% Cd-containing calcite</td>
<td>30% calcite + 40% vaterite + 30% organic Ca and/or ACC</td>
<td>Substituted calcite + substituted vaterite + W</td>
<td>Angular edges</td>
</tr>
<tr>
<td>CdG13</td>
<td>No Cd signal</td>
<td>N.m.</td>
<td>No μ-XRD peak</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdG14</td>
<td>No Cd signal</td>
<td>N.m.</td>
<td>No μ-XRD peak</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdG15</td>
<td>N.m.</td>
<td>N.m.</td>
<td>No μ-XRD peak</td>
<td>N.o.</td>
</tr>
<tr>
<td>CdG16</td>
<td>N.m.</td>
<td>N.m.</td>
<td>No μ-XRD peak</td>
<td>N.o.</td>
</tr>
</tbody>
</table>

a Sarret et al. (2007). ACC, amorphous calcium carbonate (e.g., cystolith). W, whewellite (CaC2O4·H2O). A, aragonite (CaCO3). (t), trace amounts. N.m., not measured; N.o., not observed. CdCaG1, CdCaG7, CdCaG12 and CdG12 were named CdCa3, CdCa2, CdCa6, and Cd10, respectively, in Sarret et al. (2007).
ulating the excretion of Cd-containing grains with a Ca supplement during tobacco growth and enforcing their removal during cigarette manufacturing could help decrease Cd poisoning. Tobacco is also a good candidate for the phytoextraction of metals from contaminated solid matrices due to its high biomass, fast growth, and ease of harvesting (Keller et al., 2003). Choi et al. (2001) estimated that a tobacco plant exposed in vitro for three weeks to 0.2 mM Cd and 30 mM Ca can excrete 4.2 μg of Cd. When plants were exposed for one month, the total Cd content in unwashed leaves with trichomes was 6.3 μg per plant, which suggests that the elimination of grains by trichomes is an effective mechanism of Cd detoxification (Choi and Harada, 2005). However, the application of this process to phytoextraction requires harvesting grains from the plants, otherwise their fall on the ground and subsequent acidic dissolution would release cadmium.

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REFERENCES


