Reduction of cadmium availability to tobacco (*Nicotiana tabacum*) plants using soil amendments in low cadmium-contaminated agricultural soils: a pot experiment

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Abstract

Cadmium (Cd) concentration in field-grown tobacco leaves usually ranges from <0.5 to 5 mg Cd kg^{-1} dry matter (DM). Reducing bioavailability of soil Cd by adding amendments to the soil could be suitable to mitigate Cd uptake by tobacco plants. However, little is known on the effect of inorganic amendments on agricultural soils with low Cd concentrations. Therefore, we performed a pot experiment with tobacco plants that were grown during 56 days in two neutral to alkaline agricultural soils with low total Cd concentrations (soil 1 = 0.4, soil 2 = 0.7 mg kg^{-1}). Both soils were amended or not with 1 or 5% of sepiolite, zeolite, hydroxyapatite and apatite II™. Major and trace elements were measured in mid-stalk position leaves. Soil metals were measured in a DTPA soil extraction to assess the effect of the amendments on metal bioavailability. Some amendments significantly reduced Cd concentration in tobacco leaves, but the effect differed between the two soils tested. In soil 1, the use of zeolite at the 1% dose was the most efficient, reducing the average Cd concentration from 0.6 to 0.4 mg kg^{-1}. In soil 2, the 5% hydroxyapatite treatment led to the maximal reduction in Cd concentration (50%), with an average final Cd concentration in leaves of 0.7 mg kg^{-1} (control: 1.5 mg kg^{-1}). There was a dose effect for some amendments in soil 2 (containing more Cd), suggesting a reduced efficiency of the amendment at the lowest addition rate. DTPA extractable Cd and Zn measured at the end of the pot experiment were correlated to the metal concentrations in tobacco leaves suggesting that (1) the reduction in leaf Cd concentration was due to a reduction in metal availability to tobacco and (2) DTPA may be a suitable extractant to estimate Cd availability to tobacco plants in these two soils. In addition, a batch experiment was performed with the same soils to test a larger number of amendments, including the four tested in the pot experiment. Results were compared to those of the pot experiment to assess whether a batch experiment may predict the efficiency of an amendment on a given soil. It gave results compatible with those from the pot experiment except for the sepiolite and highlighted the broad range of potential amendments available for heavy metal remediation in crop plants.

Introduction

Cadmium (Cd) concentration in agricultural soils typically results either from contamination

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due to mining, smelting of metal ores, power stations, heating systems, waste incinerators, urban traffic, cement factories and application of phosphate fertilizers and sewage sludge (Alloway, 1995; di Toppi and Gabbrielli, 1999), or may have a geogenic origin (Alloway, 1995; Baize and Sterckeman, 2001; di Toppi and Gabbrielli, 1999; Dubois et al., 1998). Even when concentrations in soil are low, Cd may be readily taken up by plant roots and translocated into aerial organs where it can accumulate to fairly high levels, representing the main source of contamination for humans through the food chain and smoking (WHO, 1992; Wagner, 1993). Food-borne Cd is the major source of exposure for most people. In most areas not polluted with Cd, average daily intakes from food are $10-40$ μg, but in polluted areas the value has been found to be several hundred μg per day. In non-polluted areas, uptake from heavy smoking may equal Cd intake from food (WHO, 1992).

Cadmium concentrations observed in plants are generally between 0.03 and 20 mg kg^{-1} and the critical range for phytotoxicity is usually $5-10$ mg Cd kg^{-1} dry matter (DM) (Sauerbeck, 1989). The value set by several countries for consumption of leafy vegetables is 0.1 mg Cd kg^{-1} fresh weight (Adriano, 2001). Tobacco (Nicotiana tabacum) can accumulate Cd at relatively high levels compared to other species (e.g. Mench et al., 1989; Wagner, 1993) and concentrations in field-grown tobacco leaves usually range from <0.5 to 5 mg Cd kg^{-1}, although higher values can also be found (Lugon-Moulin et al., 2004).

Different actions can be undertaken in order to reduce the absorption of Cd by tobacco and other plants (Lugon-Moulin et al., 2004). Metal immobilisation by addition of specific amendments to the soil may represent a potentially rapid and rather inexpensive way to prevent excessive Cd uptake by tobacco plants. Amendments or ameliorants have long been used to improve the quality of agricultural soils (organic matter, lime). When dealing with mitigation of metal uptake however, most of the research has focused on remediation of highly heavy-metal contaminated soils in order to re-establish a vegetation cover and subsequently reduce wind and water erosion and contamination of the sur-

roundings (e.g. Vangronsveld and Cunningham, 1998). Different materials, natural or synthetic, organic or inorganic, have been tested with success to reduce Cd availability to plants. These materials include phosphate (P) compounds like rock phosphate (Basta et al., 2001), apatite, hydroxyapatite (Boisson et al., 1999; Chlopecka and Adriano, 1997; Knox et al., 2001, 2003), iron (Fe) and manganese (Mn) oxides and oxyhydroxides, and Fe/Mn-bearing amendments (Didier et al., 1993; Mench et al., 1994; McBride and Martinez, 2000; Sappin-Didier and Gomez, 1994; Sappin-Didier et al., 1997), organic amendments (Jamode et al., 2003; Walker et al., 2003, 2004), inorganic clay materials including micas (illites), vermiculites, 2:1 phyllosilicates modified or not, zeolites (Chlopecka and Adriano, 1997; Gworek, 1992; Knox et al., 2001), and sepiolite (Alvarez-Ayuso and Garcia-Sanchez, 2003). Various locally available industrial by-products may advantageously replace natural materials. They include beringite (Vangronsveld et al., 1996), red mud (Lombi et al., 2002, 2003), sterilized crushed bone meal (Hodson et al., 2000), red gypsum, phosphogypsum and dolomitic residue (Illera et al., 2004), and liming agents (although liming alone cannot be considered as a suitable long-lasting treatment for metals stabilisation but rather as an enhancer of other amendment effects) (Knox et al., 2001; Tsadilas et al., 2005). Amendments may also be combined (Brown et al., 2003) and, with the exception of the organic amendments, they are usually applied at rates ranging from 0.5% to 5% DM.

In spite of successful results, some factors may limit the efficiency of these additives. Natural compounds are usually less expensive than synthetic ones, but their availability depends on the source. Moreover, their composition and purity − and thus efficiency − may vary with their origin. Organic amendments are low cost adsorbents, readily available and relatively inexpensive, but their properties may vary from one batch to another, according to the sludge-processing (Merrington and Smernik, 2003; Richards et al., 2000) and, the nature of their inorganic fraction that is partly responsible for their immobilisation capacity (Brown et al., 1998). Their efficiency may also decrease with time through decomposition of the organic matter (McBride, 1995). Phosphate

compounds affect not only heavy metal concentrations in plants, but also essential micronutrients: increasing addition of apatite to the soil has been found to decrease P, Mn and Fe concentrations and induce elemental deficiency in plant tissues (Grant and Bailey, 1993), and enhance desorption of arsenic (As) in soil (Boisson et al., 1999) and, As, selenium (Se) and thorium (Th) in sediments (Kaplan and Knox, 2004). Excess of soil phosphate may pose an environmental concern in areas with surface waters sensitive to nutrient inputs. Application of Fe and Mn-rich compounds have resulted in contradictory results (Boisson et al., 1998; Hartley et al., 2004; McBride and Martinez, 2000; Sappin-Didier et al., 1997). There have been cases where liming agents were not efficient at reducing Cd uptake (Li et al., 1996). In addition, lime must be periodically re-applied to keep soil pH at the desired level and the pH at which metal inactivation is best may be too high for optimal availability of essential nutrients. The reasons for these side effects are the nature of the amendments and the various mechanisms by which amendments alter the forms of metal contaminants in soil. These mechanisms are not fully understood and, depending on the soil and the amendment characteristics, may include precipitation, co-precipitation, surface complexation, humification, diffusion in micropores, sorption and redox transformations (e.g. Benedetti et al., 1996; Brümmer et al., 1988; Hiller and Brümmer, 1997; Kinniburgh et al., 1999; Koopal et al., 2001; Papadopoulos and Rowell, 1988; Randall et al., 1999; Skjemstad et al., 1998; Stipp et al., 1992; Spadini et al., 2003; Zachara et al., 1991).

In summary, an ideal amendment should be rapidly effective and long lasting, prevent leaching, and reduce metal bioavailability and plant uptake. In addition, it should be inexpensive, easy to handle and apply, safe for workers, compatible with and non-toxic to the plants, available and/or easy to produce, and not causing additional environmental impact to the site (Vangronsveld and Cunningham, 1998). If possible it should also offer benefits to the plants (e.g. supplying plant nutrients or increasing soil moisture holding capacity). So far, however, no real assessment of the various amendments effects, advantages and drawbacks has been performed and evaluation of the amendment efficiency relies on a site-to-site (or soil-to-soil) approach. In addition, the feasibility of the stabilisation technique on agricultural soils containing low Cd concentrations has not been sufficiently investigated (Sterrett et al., 1996).

The principal aim of this project was thus to test whether a variety of amendments were efficient at reducing Cd concentration in tobacco leaves of plants grown in low Cd contaminated agricultural soils without modifying the general nutrient status of the plants. Two complementary experiments − a pot experiment with tobacco plants and a batch experiment − were set up in controlled conditions. In the pot experiment, we tested four amendments on two soils with low Cd concentrations and measured concentrations of Cd and some essential elements in tobacco leaves. An assessment of available Cd in soil was obtained by a chemical soil extraction with diethylenetriaminepentaacetic acid (DTPA). In the batch experiment, we tested a larger number of amendments on these two soils and determined whether a batch experiment could help to predict the effect of amendments on DTPA-extractable Cd as measured in pots.

Material and method

The soils

Two soils were collected in Switzerland in the vicinity of Payerne (soil 1) and at Rafz (soil 2). Soil 1 is an agricultural soil regularly used for tobacco production. Soil 2 is an agricultural soil that was amended with municipal and industrial sewage sludge and, as a result, is contaminated with copper (Cu), zinc (Zn) and lead (Pb) (Table 1). Both soils are neutral to slightly alkaline, the carbon content is in the range usually found for agricultural soils and the total Cd concentration measured by X-ray fluorescence (XRF) is low (Table 1). The soil characteristics are favourable to low metal availability to plants and DTPA-extractable Cd, Zn, Cu and Pb concentrations are all low. The results are compiled in Table 1 and more data on soil 2 can be found in Krebs et al. (1999) and Kayser (2000).

Table 1. Selected characteristics of soil 1 (Payerne) and soil 2 (Rafz)

	Soil 1	Soil 2
pH	7.7	6.8
Org. C (%)	1.6	1.9
Inorg. C (%)	1	0.2
Clay (%)[a]	18	15
Slit (%)[a]	39	31
Sand (%)[a]	43	54
Total Cd (mg kg^{-1})	0.4	0.7
Total Cu (mg kg^{-1})	27	54
Total Zn (mg kg^{-1})	62	505
Total Pb (mg kg^{-1})	20	362
DTPA-extractable Cd (μg kg^{-1})	57	167
DTPA-extractable Cu (mg kg^{-1})	1.9	6.5
DTPA-extractable Zn (mg kg^{-1})	0.8	31.8
DTPA-extractable Pb (μg kg^{-1})	0.9	32

[a]According to Krebs et al. (1999).

The amendments

Several amendments belonging to different categories were tested: (1) sepiolite ($Mg_4Si_6O_{15}(OH)2 \cdot 6\text{-}H_2O$) is a magnesium silicate hydroxide hydrate mineral, commercialised as PANSIL−100 by TOLSA SA (Madrid, Spain), (2) a natural zeolite (Ø1−2.5 mm), purchased from Unipoint AG, Truttikon (CH), (3) a synthetic hydroxyapatite (calcium phosphate tribasic, $Ca_{10}(OH)_2(PO_4)_6$), purchased from Alfa Aesar (Karlsruhe, Germany), (4) apatite II™ (thereafter referred to as apatite II), a commercially available form of apatite derived from fish bones and purchased from Slater (UK) Ltd (United Kingdom), (5) a finely ground natural vermiculite (cation exchange capacity of 900−1000 mmol kg^{-1}) purchased from Vermica AG (Bözen, CH), (6) an industrial compost (Brill® purchased from OBI, CH) made of 96% peat, 4% Perlite, and containing traces of AGROSIL®, guano, lime and nutrients, (7) a commercial charcoal from Swiss hardwood (Migros, CH) ground in a ceramic mortar at <100 μm (this charcoal was found to fix large amounts of Cd; Hammer et al., unpublished), (8) pure lime, a calcite ($CaCO_3$) that was obtained from Merck (No. 2064), (9) a cement flour, which is a mixture of pure calcium carbonate with marl, produced at the Eclepens cement industry (sample Q10111, collected the 2nd July 2003, Holcim, CH) and ground and sampled before heating, (10) goethite (α-FeOOH) supplied by Ward's Natural Science Establishment Inc. (USA) as a row material and ground at <100 μm prior to use. These amendments were chosen either for their efficiency at reducing Cd or other metals uptake in (often) highly contaminated soils (hydroxyapatite, apatite, zeolite, goethite, vermiculite, compost, lime) or their potential efficiency at binding Cd (apatite II, sepiolite). They were also selected for their commercial availability (sepiolite, apatite II, zeolite, hydroxyapatite, cement flour, lime, compost). In some cases, pure materials were used (calcite, hydroxyapatite) to obtain maximum efficiency. In addition, because tobacco is produced worldwide, the amendments may need to be transported over large distances and thus low addition rates were preferred. Amendments that would have required large application rates like biosolids were not selected for the pot experiment.

Total element analysis was performed on finely ground samples (ceramic mortar Retsch RM 100) by XRF as for the soils and selected results are presented in Table 2. Cadmium concentrations are below detection limit except in goethite, sepiolite, lime, cement flour and hydroxyapatite. However, Cd is most likely included in the lattice of the components as impurity and thus not readily available for plants. In addition, the application at 5% addition rate of one amendment containing 1 mg Cd kg^{-1} would increase the soil total concentration of 0.05 mg kg^{-1}.

Protocol of the pot experiment

Experimental design

The pot experiment was conducted on both soils with sepiolite, zeolite, hydroxyapatite and apatite II at 1 and 5% addition rates (calculated on a soil dry weight basis). Similar addition rates resulting in reduction of Cd concentration in plants have been commonly reported in the literature (Boisson et al, 1999; Chlopecka and Adriano, 1997; Gworek, 1992; Mench et al., 1994; Sappin-Didier and Gomez, 1994; Sterrett et al., 1996). Soils were passed through a 1-cm sieve. Amendments were added and thoroughly mixed by hand. The pots were filled with 4 kg of soil 1 and 3.5 kg of soil 2 (difference due to different initial moisture content). All treatments were performed in triplicates. Three control pots per soil were also set up without amendment. Pots were watered and

Table 2. Selected element concentration in the various amendments used in the pot and batch experiments (XRF analysis)

Amendments	Mg (%)	Al (%)	Si (%)	P (%)	K (%)	Ca (%)	Fe (%)	Cd (mg kg^{-1})	Cr (mg kg^{-1})	Ni (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	As (mg kg^{-1})	Pb (mg kg^{-1})
Sepiolite	9.72	1.98	23.8	0.02	0.9	1.13	1.1	0.5	28	7	10	35	4	14
Zeolite	0.35	4.83	29.5	bdl[a]	2.57	2.01	0.9	bdl	10	1	13	27	0.8	10
Hydroxyapatite	0.32	0.08	0.3	16.4	bdl	39.4	0	0.9	12	bdl	5	17	1.1	1
Apatite II	0.23	0.06	0.1	7.07	0.24	17.7	0	bdl	5	bdl	2	73	0.7	2
Vermiculite	12.2	3.93	17.7	0.07	4.09	0.79	5.6	bdl	244	165	24	77	bdl	2
Compost	0.12	0.21	0.6	0.08	0.22	2.81	0.2	bdl	7	2	15	19	2.8	9
Charcoal	nd[b]	nd	nd	0.04	0.38	0.83	0	bdl	4	1	6	35	bdl	2
Lime	bdl	0.01	0.1	bdl	bdl	42	0	0.6	bdl	bdl	3	3	bdl	2
Cement flour	0.59	1.92	6	0.01	0.57	30.4	1.5	0.5	75	48	24	115	8.4	66
Goethite	0.14	1.14	2.6	0.24	0.44	0.04	52.5	1.2	111	173	50	357	140	66

[a]bdl: below detection limit; [b]nd: not determined.

stored in a climate chamber, where they were left to settle a minimum of 6 weeks at room temperature before transplanting the tobacco plants.

Plant cultivation and sampling

We used the French variety ITB 33024 (Virginia), which resists black root rot (*Chalara elegans*), as the soils used for the pot experiment may have contained this pathogen. Seeds were sown in a metal-free substrate (peat or compost). The plants were watered regularly by overhead irrigation and fertilised once per week with WUXAL® Engrais Universel (N 100 g L^{-1}, P$_2$O$_5$ 100 g L^{-1}, K$_2$O 75 g L^{-1}, and B, Cu, Fe, Mn, Mo, Zn) at 0.2%. The trays were set up in a climate chamber (day/night period 16/8 hours, day/night temperatures 20 °C/18 °C, 22 °C after 4 weeks and 24 °C after two more weeks, light intensity of 500 lux).

When the plants had developed three pairs of leaves, they were transferred to the pots. They were then left to grow in the climate chamber during 56 days (day/night temperatures 24 °C/20 °C), with regular watering and random rotation of pots position. All the plants (except those growing in the soils with 5% apatite II) presented chlorosis symptoms on the lower leaves after about three weeks of growth in pots. They were then treated with a fertiliser once per week with a homemade nutrient solution (mixture of 1 g Ca(NO$_3$)$_2$·4H$_2$O, 0.25 g MgSO$_4$·7H$_2$O, 0.25 g KCl, 0.25 g KH$_2$PO$_4$ per litre) that alleviated the symptoms. Morphology and visual symptoms were recorded after 55 days of growth. After 56 days, all plants were harvested. Some of the plants had reached the flowering stage, others had not. As Cd accumulates less in the lower leaves than in the medium and upper ones (Lugon-Moulin et al., 2004), leaves were grouped by 4 (foliar stages), starting from the bottom of the plant. Only leaves from the second stage (hereafter referred to as mid-stalk position leaves) were analysed. The plant samples were oven-dried at 60 °C during one week and the total dry weight of the aerial parts was recorded.

Soil sampling

Soil samples representative of the rhizospheric soil (soil adhering to the roots after shaking of the root system) were taken from all pots after tobacco harvest. Practically, virtually no bulk soil was left. The samples were oven-dried at

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40 °C until constant weight, sieved at 2 mm and extracted with DTPA (see below).

Batch experiment

All 10 amendments were tested in batch experiment in duplicates and at a single addition rate of 3%. The 2 initial soils were oven-dried at 40 °C until a constant weight was reached, ground and sieved at 2 mm. The finely ground amendments were added to the soil in 50 mL polyethylene (PE) flasks and humidified so that there was no water settling at the bottom of the flasks. They were let to oven-dry at 40 °C until dryness was reached (3 or 4 days). The samples were then re-moistened. The process was repeated during 1 month twice per week (eight repeated wet-and-dry cycles), in order to mimic an accelerated aging process. After the last drying, samples were extracted with DTPA (see below) in the same flasks in order to prevent soil loss and contamination.

Analytical methods

Soil analysis
Initial soil samples were dried at 40 °C, and 2 mm-sieved samples were analysed for pH (0.01 M $CaCl_2$) (FAL, 1998). Sub-samples were further ground to 100 μm in a ceramic mortar (Restch RM 100, Germany) and analysed for organic and inorganic carbon and total elements by XRF (Spectro X-Lab2000 Kleve Germany).

DTPA-TEA extraction (0.005 M DTPA, 0.1 M triethanolamine and 0.01 M $CaCl_2$ at pH 7.3; soil/solution 1/2; horizontal shaker 120 min, centrifugation and filtration on Schleicher & Schuell 0.45 μm membrane; Schleicher & Schuell, Dassel, Germany), as proposed by Lindsay and Norvell (1978), was performed on 2 mm-sieved samples to assess the metal DTPA-extractable fraction in the initial soils and both in the pot experiment after harvest of the tobacco plants and in the batch experiment after the wet-and-dry cycles. The DTPA extraction was chosen after preliminary comparative tests including DTPA, EDTA (ethylenediaminetetraacetic acid)-NH$_4$OAc at pH 4.65, 1 M NH$_4$NO$_3$ and 1 M NH$_4$OAc. DTPA was found to be the most suitable owing to the soil pHs and because Cd concentrations in the two latter extractants were below the detection limit and did not discriminate between the soils.

"Initial soil" always refers to soil samples analysed before any experiment. "Control soil" refers to soil samples collected at the end of the pot experiment, in pots grown with tobacco and without amendment. "Treated soil" is similar to "control soil" but with amendment.

Plant analysis
Finely ground plant samples (0.5 g) were digested in 8 mL HNO$_3$ 65% (Fluka, Buchs, Switzerland) and evaporated. The residues were re-dissolved in 1 mL HClO$_4$ 70% (Fluka, Buchs, Switzerland) and heated 1 h to 235 °C before dilution to 10 mL with purified water (modified from Keller et al., 2003).

Elemental analyses
Cadmium and the other elements were measured in soil extracts and plant digests by Inductively Coupled Plasma-Atomic Emission Spectrophotometry (ICP-AES) (Plasma 2000; Perkin Elmer, Wellesley, USA). All extractions and digestions were performed using p.a. or suprapur products as required. Deionised water (MilliQ) was used throughout the various extractions. All plant and soil samples were run together with certified reference material (sample W921, Wageningen, NL) for soil or in-house standard for plant to assess the accuracy of the measurements.

Statistical analysis
Analyses of variance (ANOVA) were performed to test the soil, the amendment and the dose effects on element concentrations in plants as well as on biomass production (ANOVA, SYSTAT 10.2, SYSTAT Software Inc.). The effect of a given factor was considered significant when the P-value was <0.05. In addition Student's t-tests were performed to test whether the average concentrations in leaves of tobacco grown in the treated pots differed from those of tobacco grown in the control pots. The difference between means was significant (labelled * in the figures) when $P < 0.05$. Pearson's correlation coefficients were calculated between element concentrations measured in plants and in DTPA extracts (pot experiment), either combining the two soil datasets or for each soil independently.

The significance was: *** for $2\alpha < 0.001$, ** for $2\alpha < 0.01$ and, * for $2\alpha < 0.05$.

Results

The pot experiment

Biomass production
The overall chlorosis that developed on all plants after about 3 weeks of growth in pots (except on plants growing on 5% apatite II) was probably due to a nutrient depletion. It was remediated by the addition of fertiliser. Plantlets transplanted to pots with 5% apatite II were not able to establish. Most of them immediately died and had to be replaced several times. The growth of the plantlets that ultimately established was highly variable (large SD). For this treatment the growth period was shorter (all plants were harvested at the same time) and resulted in an under-estimated biomass production at harvest.

The type of soil as well as the treatment had an influence on biomass production (ANOVA, soil effect: $F = 53.4$, $P < 0.001$; amendment effect: $F = 13.8$, $P < 0.001$), but there was no significant soil × amendment interaction ($F = 1.06$, $P = 0.41$). Control plants on soil 2 produced about twice as much biomass as those on soil 1, whereas plants in the treated soil 2 produced on average 50% more biomass than those in the treated soil 1 (Figure 1). Plants grown on 1% apatite II were the exception, with a similar biomass on both soils.

There were no significant differences between the 1 and 5% treatments, except for apatite II (see below). In soil 2 the difference between 1 and 5% sepiolite was only marginally significant ($P = 0.066$). The biomass in the 5% apatite II treatment was halved in soil 2 and divided by four in soil 1 when compared to the 1% dose. This large difference was probably due to the difficulty for plants to establish and is not thus directly comparable to the other treatments, but it shows that 5% apatite clearly impacted plant growth. In addition, the morphology of tobacco plants growing in pots with apatite II (both treatments) was different from that of the other plants, with larger leaves, no chlorosis symptoms and thicker stems.
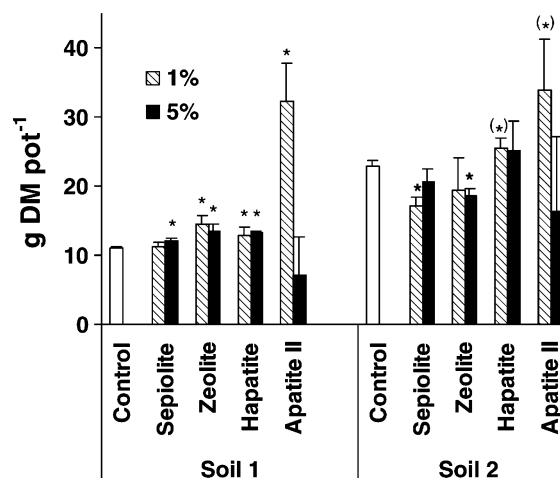

Figure 1. Dry shoot biomass produced by tobacco plants grown 56 days in pots, according to the soil and the amendment; *means significance of Student's t-test at $P < 0.05$ and (*) at $P < 0.1$.

Effect of treatments on nutrient concentrations in plants
Figures 2 and 3 give the results of elemental concentrations in plants for some major and trace elements. The soil type as well as the amendments had a significant effect on P, magnesium (Mg) and calcium (Ca) concentrations in tobacco leaves (two-way ANOVA, all $P < 0.001$), but not on Fe concentrations. However, the effect of amendments on P was no longer significant when apatite II was excluded from the calculations. The soil × treatment interaction was significant neither for Mg, Ca and Fe, nor for P when the apatite II treatment was removed from the analyses. In both soils, Ca concentrations were reduced in tobacco grown in the sepiolite and zeolite treated pots when compared with the control pots, but a significant increase was observed in the pots treated with apatite II. The effects of the different treatments on Mg and P concentrations differed between the 2 soils and according to the amendment and could either lead to an increase or a decrease of Mg and P concentrations.

Effect of treatments on Cd and other metals concentrations in plants
There was a significant soil effect on the concentrations of Cd, Zn and Pb (ANOVA, all $P < 0.001$). For each of them, as well as for Cu, there was also an amendment effect (ANOVA, $P < 0.001$ for Cd, $P = 0.043$ for Zn, $P = 0.015$
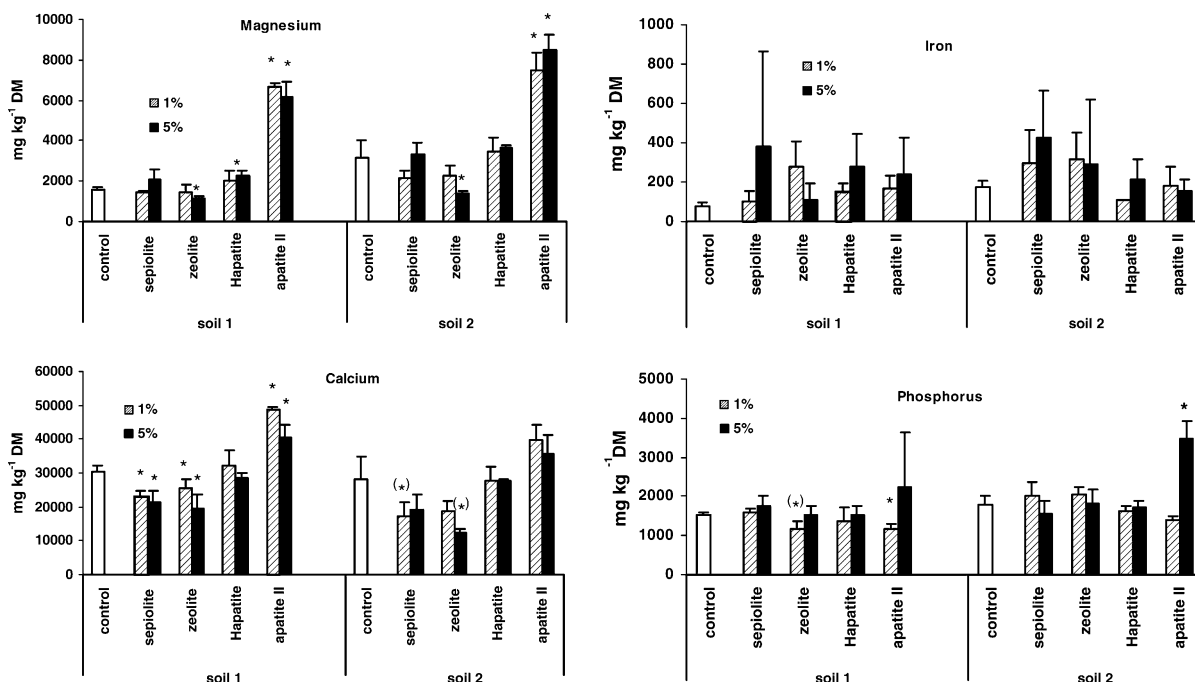
Figure 2. Magnesium, Ca, Fe and P concentrations in the mid-stalk position leaves of tobacco grown in soil 1 (Payerne) and soil 2 (Rafz) with and without amendments; *means significance of Student's *t*-test at $P < 0.05$ and (*) at $P < 0.1$; Hapatite = hydroxyapatite.
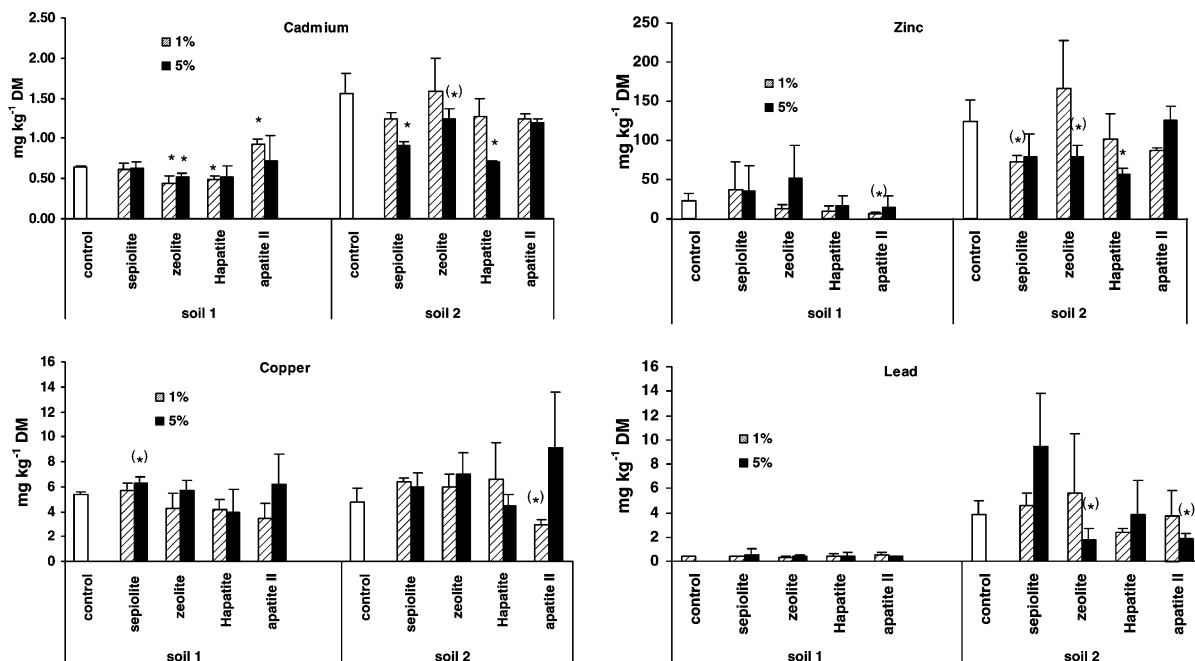

Figure 3. Cadmium, Zn, Cu and Pb concentrations in the mid-stalk position leaves of tobacco grown in soil 1 (Payerne) and soil 2 (Rafz) with and without amendments; *means significance of Student's *t*-test at $P < 0.05$ and (*) at $P < 0.1$; Hapatite = hydroxyapatite.

for Cu and, $P = 0.036$ for Pb). However, for Cu and Pb it could either lead to an increase (5% sepiolite in soil 1 and 1% zeolite in soil 2) or a decrease (1% apatite in soil 2) in concentration.

Cadmium concentrations were higher in tobacco grown on the sewage sludge-contaminated soil (soil 2) than in plants grown on the uncontaminated soil 1. A significant decrease in Cd concentration was observed with both addition of zeolite (*t*-test, $P = 0.020$ for 1% and $P = 0.028$ for 5%) and 1% hydroxyapatite ($P = 0.016$) in soil 1 and, with 5% sepiolite (*t*-test, $P = 0.046$) and 5% hydroxyapatite ($P = 0.030$) in soil 2. In this soil, 5% zeolite gave a marginal decrease in Cd concentration ($P = 0.123$). The large standard deviation measured in the control pots was probably responsible for the non-significance of Cd concentration reduction in other treatments. There was also a significant interaction between soils and amendments (ANOVA, $F = 6.86$, $P < 0.001$). The largest reduction in Cd concentration amounted to 33% in soil 1 (1% zeolite) and 50% in soil 2 (5% hydroxyapatite). There was a significant dose effect in soil 2 (ANOVA, $F = 25.2$, $P < 0.001$), but not in soil 1. In soil 2, a 5% addition was necessary to reduce significantly Cd concentrations in plants.

Cadmium and Zn concentrations in plant leaves were positively correlated when data for the two soils were combined (Figure 4: $r = 0.745$***) and in soil 2 alone as well ($r = 0.667$***, Table 3). In soil 2, as for Cd, 5% hydroxyapatite was efficient at reducing Zn concentrations in
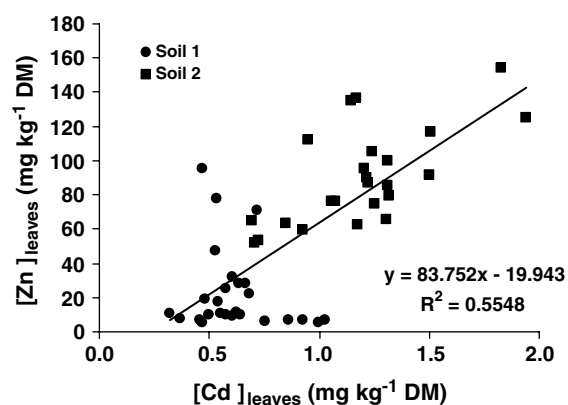

Figure 4. Linear regressions between Cd and Zn concentrations in the mid-stalk position tobacco leaves.

Table 3. Pearson's correlations between Cd concentration in mild-stalk position leaves and selected element concentrations in leaves and soils (amendment-treated and control pots)

	Soil 1		Soil 2	
Cu in tobacco	0.152	–	0.052	–
Fe in tobacco	0.062	–	0.06	–
Mg in tobacco	0.756	***	0.014	–
Mn in tobacco	0.531	*	0.05	–
Ni in tobacco	0.263	–	0.309	–
Pb in tobacco	0.125	–	0.045	–
Zn in tobacco	0.125	–	0.667	***
P in tobacco		–	0.066	–
DTPA-extractable Cd	0.278	–	0.299	–
DTPA-extractable Zn	0.405	–	0.467	*

***$2\alpha < 0.001$. *$2\alpha < 0.05$.

leaves (*t*-test, $P = 0.045$) whereas 1% sepiolite and 5% zeolite induced a marginally significant Zn reduction (respectively $P = 0.073$ and $P = 0.088$). In soil 1, all Zn concentrations, including those of the control pots, were low and close to 25 mg kg^{-1} that is set as the limit for Zn deficiency for tobacco. And although a trend could be detected, there was no significant difference in Zn concentrations between the control and treated pots, except a marginally significant reduction obtained with 1% apatite II (*t*-test, $P = 0.059$). Therefore, in the two soils tested, some of the amendments chosen for their potential ability to reduce Cd uptake appeared to be also efficient at reducing Zn uptake.

Amendments had no significant effect on Cu and Pb concentrations in tobacco leaves, except marginally 5% sepiolite for Cu (increase, *t*-test, $P = 0.059$) in soil 1 and, in soil 2, 5% zeolite ($P = 0.069$), 5% apatite II ($P = 0.073$) for Pb and 1% apatite II ($P = 0.093$) for Cu.

Effect of treatments on element concentrations in DTPA extracts

Figure 5 presents the results of the DTPA extractions performed on the rhizospheric soil collected from the pots after tobacco cultivation. Cadmium concentration in soil 1 was very low (about 20 μg kg^{-1}). Therefore, the results of DTPA extractions for soil 1 should be taken with caution. A significant decrease in soil DTPA-extractable Cd was measured with 5% sepiolite (*t*-test, $P = 0.026$) and 5% hydroxyapatite (*t*-test, $P = 0.001$) in soil 2, and with 5%
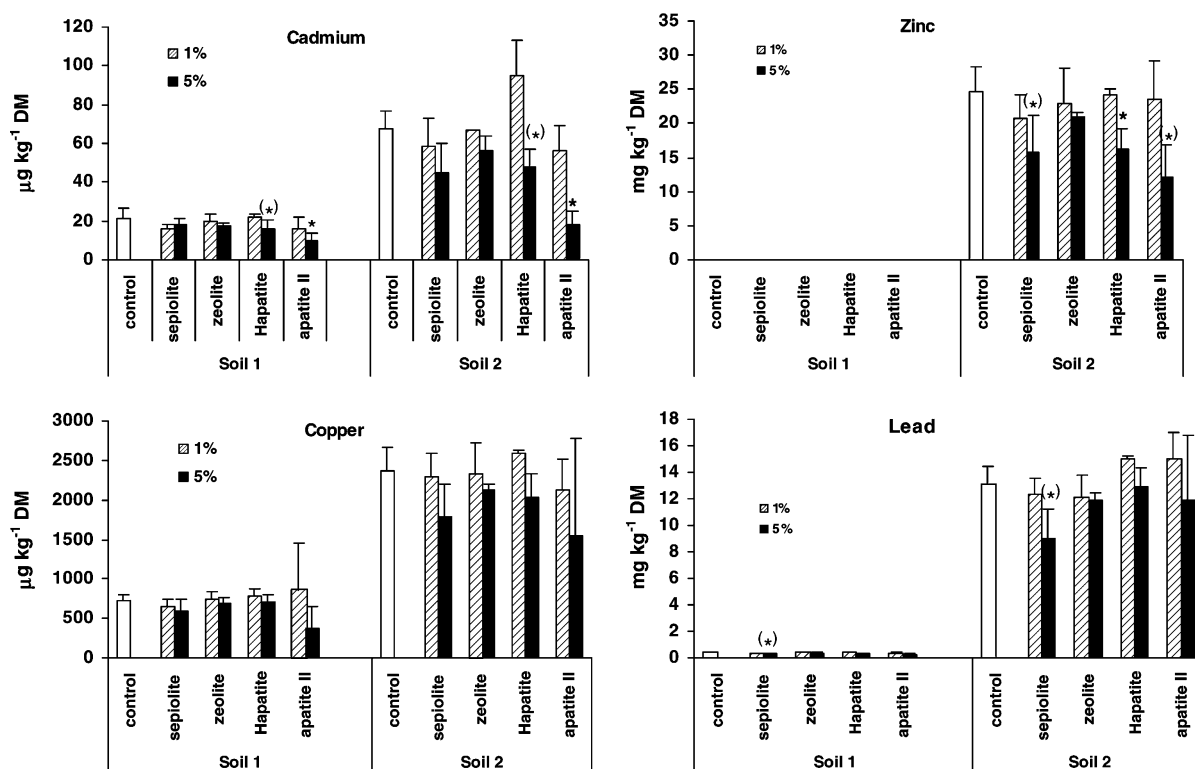
Figure 5. Concentrations of DTPA-extractable Cd, Zn, Cu and Pb performed on soils from the pot experiment after tobacco harvest; *means significance of the *t*-test at $P < 0.05$ and (*) at $P < 0.1$; Hapatite = hydroxyapatite.

apatite II in both soils (only 2 samples in soil 1 and $P = 0.039$ for soil 2). In soil 2, zeolite tended to decrease the amount of DTPA-extractable Cd, though not significantly.

As for Cd in soil 2, DTPA-extractable Zn concentrations could be significantly decreased by the addition of 5% hydroxyapatite ($P = 0.040$), whereas 5% sepiolite ($P = 0.089$) and 5% apatite II ($P = 0.097$) could decrease only marginally DTPA-extractable Zn (Figure 5; in soil 1, Zn concentrations were below detection limit). The DTPA-extractable Cu and Pb concentrations did not significantly differ between the amendment-treated and control pots, except Pb concentrations with 5% sepiolite (marginally) in both soils (Figure 5).

Relation between DTPA-extractable Cd and Cd uptake by plants
Although DTPA-extractable Cd and Cd concentrations in leaves were significantly correlated ($r = 0.744***$) when the two soils data were pooled, there was no correlation for each soil taken individually (Table 3). In soil 2, Cd concentration in leaves was also significantly correlated with DTPA-extractable Zn ($r = 0.467*$, Table 3).

The batch experiment

Figure 7 shows the amount of DTPA-extracted Cd in soil 1 and 2 after 8 wet/dry cycles, for each soil/amendment mixture. When compared with the DTPA-extractable Cd concentrations measured in the initial soils (resp. 57 and 167 μg kg^{-1} for soil 1 and soil 2, Table 1), all treatments except the sepiolite reduced DTPA-extractable Cd. The use of lime resulted in the lowest average DTPA-extractable Cd in soil 1, but average DTPA-extractable Cd concentrations were always very low in soil 1. In soil 2, vermiculite seemed the most effective amendment. Similar results were obtained for Zn and Cu (data not shown).
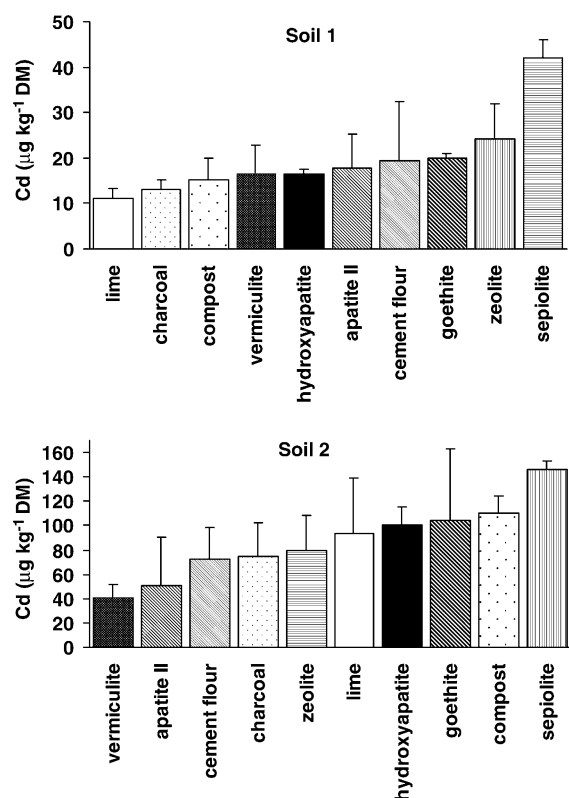
Figure 6. DTPA-extractable Cd concentrations measured in soil 1 and soil 2 after 8 dry/wet cycles in the batch experiment.
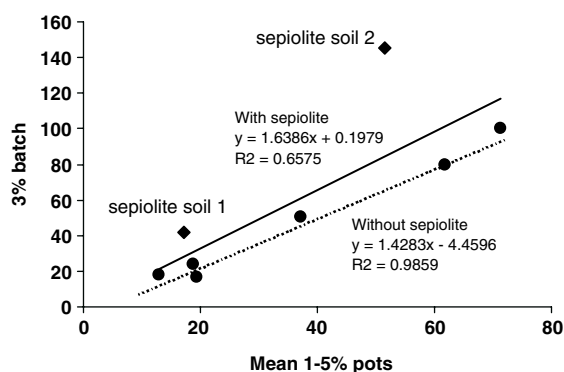

Figure 7. Relationship between average DTPA-extractable Cd concentrations measured in pot and batch soils. Solid line = linear regression with all points and dashed line = linear regression without the sepiolite points.

Relation between the batch and pot experiment results

Figure 7 shows the correlation between the results of the pot experiment (by taking the mean of the 1% and 5% dose results) and the batch

experiment (3% dose) for the DTPA-extracted Cd for soil 1 and 2, and the four amendments common to both experiments. The amount of Cd extracted from the batch samples and the amount of Cd extracted from pot soil samples were significantly correlated (Pearson's correlation coefficient $r = 0.813*$). The sepiolite treatment resulted in the highest DTPA-extractable Cd concentrations in the batch experiment (Figure 6). We performed a regression analysis without this amendment and the regression was improved ($r = 0.993***$). This result suggests that for all soil/amendments combinations except those involving sepiolite, the 8 wet/dry cycles of the batch experiment gave the same order of efficiency of the different amendments than 3–6 weeks of equilibration and 6 weeks with tobacco growing in the pots when DTPA-extractable Cd concentrations were compared. However, the amount of Cd extracted from the batch samples was always larger than the fraction extracted from the pot samples.

Discussion and conclusion

The pot experiment gave information on element uptake by plants according to the soil and the treatment (type and rate). It also allowed assessing the change in DTPA-extractable metal after harvest of tobacco grown with and without treatment.

We showed that several amendments could significantly decrease Cd concentration in tobacco leaves grown on agricultural soils with low Cd concentrations. The Cd concentrations we reported in tobacco leaves lie within the range commonly reported in field-grown tobacco (Lugon-Moulin et al., 2004). So far there has been no direct correlation made between Cd concentration in tobacco leaves and Cd intake by smokers. However, it is hypothesised that a reduced Cd content would decrease Cd in smoke. The effects of the amendments on leaf Cd concentrations were however soil- and dose-dependent. The significant interaction between soils and amendments underlines the necessity to choose the amendment according to the soil type.

Some amendments had also an effect on other element concentrations in plants. Zinc concentrations measured in tobacco grown in soil 1 were

lower than in the control pots and may have led to a Zn deficiency (below $20-25$ mg Zn kg^{-1} DW). However, Zn concentrations in control plants were already very low and no visible deficiency symptoms were specifically observed on the treated tobacco plants. In addition, no morphological difference was observed between tobacco plants grown on soils 1 and 2 when treated with the same amendment. The most significant changes were observed for Ca and P concentrations in tobacco plants grown in pots treated with apatite II. This may have resulted from the release of Ca and P contained in apatite II as well as from the different growth behaviour observed with both addition rates of this treatment compared to the other treatments.

The addition of amendments also decreased DTPA-extractable Cd, although not always significantly. The mechanisms involved in the reduction of DTPA-extractable Cd may be various and depend on both the amendments and the soil characteristics, which play an essential role in metal availability. Assessment of the changes in the Cd forms and measurement of a larger set of soil parameters would allow more insight into the mechanisms that might be responsible for Cd immobilisation.

One of the objectives of the pot experiment was also to investigate the relationship between the DTPA-extractable Cd in soils and the concentration of Cd found in tobacco growing on these soils. Practically, this could lead to an estimate of the expected concentration of Cd in crops prior to their cultivation, applying a simple technique (chemical extraction), if DTPA was able to assess correctly Cd availability to tobacco. Indeed, Cd concentrations in tobacco leaves were correlated to DTPA-extractable Cd in soil 2, or when both soil datasets were pooled. However, measurements in soil have been performed after plant harvest and hence, integrated both the amendment effect and the fraction of Cd taken up by the plant. As the total Cd incorporated into the plant amounted to only 10% of the sum DTPA-extractable Cd + Cd in plants in soil 1 and 13% in soil 2, it is likely that the reduction in Cd concentrations in tobacco leaves was directly related to the decrease in DTPA-extractable Cd in soil. Tsadilas et al. (2005) also observed a parallel decrease of DTPA-extractable

Cd and Cd concentration in Virginia tobacco leaves grown in the field after treatment of the soil with 3000 kg Ca(OH)$_2$ ha^{-1}. It also suggests that DTPA-extractable Cd may represent a soil Cd fraction available to tobacco plant in the soils we investigated. Similar results were obtained for Zn. However, published studies aiming at predicting Cd concentrations in tobacco leaves using various soil properties and Cd chemically extractable fractions suggest that the use of a single extractant like DTPA may not accurately predict Cd uptake by tobacco in all field situations (e.g. Adamu et al., 1989; Miner et al., 1997; Tsadilas, 2000) and that soil characteristics such as pH, cation exchange capacity and clay content should be included (Wang et al., 2004). This aspect has not been tested in our experiment and ought to be further developed and tested for tobacco in low Cd contaminated soils, using larger soil datasets.

Chlopecka and Adriano (1997) tested 1.5% zeolite and 0.4% apatite on a soil artificially contaminated with increasing concentrations of Cd. They found that the two amendments were efficient at reducing Cd concentrations in 3-week-old maize and in barley. Cadmium concentration in plants having initially 0.65 mg Cd kg^{-1} could be reduced by 64% with 0.4% apatite. They obtained similar results for Zn. Our results are in the same order of magnitude, although lower for Cd (respectively 33% and 40% Cd and Zn reduction for soil 1 with 1% zeolite and no effect on soil 2, and, respectively, 25% and 55% Cd and Zn reduction for soil 1 and 18% and 18% for soil 2 with 1% hydroxyapatite). In addition, they found that the amendments were more efficient when Cd concentrations in soil were low, probably because at higher soil Cd concentrations the amount of amendment added was not sufficient to immobilise all available Cd. In our case, the limited decrease in Cd concentration in plants grown on the uncontaminated soil 1 with the 1% and 5% treatment suggests that we were dealing with residual Cd concentrations in soil. As a result, the addition of 1% amendment to this soil was enough to reduce metal availability to plants. In soil 2 however, reduction in Cd concentration was larger when the 5% dose was applied irrespective of the treatment, suggesting that a 1% addition rate was not enough to

immobilise all available Cd. Larger doses may be even more efficient but not realistic. The technical feasibility will have to be assessed. The difference between our results and those of Chlopecka and Adriano (1997) may further be due to the fact that tobacco is more efficient at taking up Cd and translocating it to its leaves than maize (Mench et al., 1989; Mench and Martin, 1991). For example, Mench et al. (1994) and Sappin-Didier and Gomez (1994) found that Cd concentrations in plant shoots were always lower and the percent of reduction larger for ryegrass than for tobacco. Larger reduction in Cd uptake by plants using synthetic zeolite or phosphate compounds was usually obtained in highly contaminated soils (Boisson et al., 1999; Gworek, 1992), although Basta et al. (2001) did not find any beneficial effect of rock phosphate on Cd uptake by lettuce grown on highly contaminated soils.

Studies on Cd removal from waste water by sepiolite (Brigatti et al., 1998) and batch and columns studies performed on a contaminated soil (Alvarez-Ayuso and Garcia-Sanchez, 2003) have given promising results for Cd and Zn removal. However, no experiment had been performed to test its effect on metal uptake by plant. In our experiment, sepiolite was efficient at reducing both Cd and Zn concentrations in tobacco grown in soil 2. Its use would deserve further experimentation. Previous results obtained with apatite II™ have proved its efficiency at reducing Cd concentrations in the soil solution (Matheson et al., 2002). In our experiment, the 5% treatment induced plantlets mortality, but then produced healthier plants once successfully transplanted. Because of these ambiguous results, it is difficult to meaningfully compare these results with those of the other amendments, and further experimentation is needed before concluding on the suitability of apatite II™ for use with tobacco plants.

Because tobacco seems to be able to take up Cd efficiently, Cd concentrations in leaves can reach relatively high levels even in soil with relatively low Cd concentrations. Experiments with tobacco are scarce and have been performed on highly contaminated soils. They have shown that amendments such as steel shot (Sappin-Didier and Gomez, 1994) or hydrous manganese oxides (HMO) (Mench et al., 1994) can significantly reduce Cd concentrations in tobacco leaves (cv. PBD6). Mench et al. (1994) reported 41% and 63% reduction with 1% HMO in two different soils. Sappin-Didier and Gomez (1994) reported 16% and 40% reduction with 1% steel shot in the two soils they investigated. The differences with our results may be due to factors like the different amendments and soils used, and differences in DTPA-extractable Cd in soil. As already mentioned, DTPA-extractable Cd concentrations in our 2 soils were low, and control tobacco leaves contained 0.6 and 1.5 mg Cd kg^{-1}. In the studies of Mench et al. (1994) and Sappin-Didier and Gomez (1994), control tobaccos contained ca. 40 and 120 mg Cd kg^{-1}, depending on the soil. It has also been reported that Cd uptake and subsequent tissue partitioning in tobacco may vary with Cd exposure, and different varieties may respond differently (Lugon-Moulin et al., 2004). This should also be kept in mind when comparing results across studies.

The aim of the batch experiment was (1) to test a larger number of amendments in soil 1 and 2 for the reduction of DTPA-extractable Cd and, (2) to assess the ability of a batch experiment to predict the effect of an amendment on Cd uptake by plants by comparing the results with those of the pot experiment. The batch and pot experiments gave similar results for each amendment, except for sepiolite in soil 2. In the pot experiment, the reduction in DTPA-extractable Cd concentration was between 0% and 45% when compared to control pots (with tobacco), but reached 57−78% when compared to the initial soil data. In the batch experiment, the reduction was between 40% and 70% (except sepiolite which gave a, respectively, 26% and 13% reduction for soil 1 and 2) when compared to the DTPA-extractable concentrations in the initial soils. The difference observed between the two experiments when final DTPA-extractable concentrations were compared (Figure 7) may be due both to a difference in the length and protocol of "aging" of the soil/amendment mixture and plant uptake that also contributed to the decrease of the amount of DTPA-extractable Cd in the pot experiment. However, in spite of a high correlation between the pot and the batch experiments, it is not possible yet to

use the results of the batch experiment to choose with accuracy the best amendment for a given soil. This correlation will have to be confirmed and refined using a larger soil and amendment dataset.

The batch experiment further suggested that other amendments may be suitable for reduction of DTPA-extractable Cd, but their effects may vary according to the soil type. However, there were only two replicates per treatment and they presented important variations. Therefore, at this point, amendments cannot be securely classified according to their efficiency to reduce the DTPA-extractable pool of the two investigated soils.

There are potentially a large number of amendments that may be efficient at reducing Cd concentration in tobacco leaves, depending on soil type. Synthetic compounds are usually more efficient because they are purer than natural ones. Pitcher et al. (2004) found that synthetic zeolites were more efficient than natural ones at removing Zn, Cd, Cu and Pb from motorway stormwater. In our pot experiment this may partially explain the good efficiency of hydroxyapatite at reducing Cd uptake by tobacco plants. However, the price, availability and composition of synthetic compounds may be the main limitation to their use on a large scale. Our aim was indeed to test easily available and cheap amendments to be used worldwide because commercial tobacco growing is performed on all continents (except Antarctica). This precluded synthetic amendments and orientated our choice towards natural and common compounds. For other reasons biosolids, which are known to achieve significant reduction in Cd availability to plants (Sterrett et al., 1996; Basta et al, 2001; Walker et al., 2003), were not tested in pots. Indeed, their efficiency may vary largely from one place to another and with time because of variation in their composition or processing, requiring a systematic and permanent quality control. Moreover, the origin of their sorption efficiency is not clearly established (Merrington and Smernik, 2003) and might decrease with time through decomposition of the organic matter (McBride, 1995), although the inorganic residue is also often claimed to be the active immobilising fraction in biosolids (Brown et al., 1998). In addition, owing to the large amounts needed, transport over large distances may not be cost efficient.

Keeping this in mind, soil Cd immobilisation using soil amendments appears to be an easy, relatively inexpensive and flexible approach that may be suitable for application in agricultural soils with low Cd concentrations. This approach would deserve further in depth research in order to adapt it to the large panel of soils cultivated with tobacco worldwide.

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