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# Phytomanagement of metal-contaminated agricultural land using sunflower, maize and tobacco

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#### ABSTRACT

We investigated the long-term effectiveness of phytomanagement (the combination of profitable crop production with the gradual reduction of soil contamination by phytoextraction) to deal with moderately metal-contaminated agricultural land. In a 6-year field experiment, we grew maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.) and tobacco (*Nicotiana tabacum* L.) in crop rotation. The addition of elemental sulphur (2136 kg ha<sup>-1</sup> yr<sup>-1</sup>) decreased the soil pH from 7.4 to 6.7, increased the Zn accumulation by maize, sunflower and tobacco by factors of 1.3, 1.4 and 1.2, respectively, and increased the Cd accumulation by tobacco 1.3-fold. Neither the addition of ammonium sulphate (129 kg ha<sup>-1</sup> yr<sup>-1</sup>) nor nitrilotriacetic acid (NTA, 430 kg ha<sup>-1</sup> yr<sup>-1</sup>) significantly increased phytoextraction. The results show that phytoextraction for soil cleansing would require centuries. However, this land could be used to generate profitable crops, including the production of safe (low Cd) stock fodder fortified with Zn, green manure for micronutrient-deficient soils, or bioenergy.

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## 1. Introduction

Excessive application of low-quality fertilizers, pesticides, sewage sludge and other bio wastes has increased the concentrations of heavy metals in many agricultural soils worldwide above levels considered safe for food production. Metal leaching from contaminated soil into waters and transfer into food chains can endanger human health as well as ecosystem quality. Phytoextraction, i.e. the use of plants to extract contaminants from soils (Salt et al., 1995), is often touted as a gentle, environmentally friendly cleanup method. Theoretically, it should be suited for the treatment of large areas of agricultural land that are contaminated at low to medium levels (McGrath et al., 2002). Repeated cropping of plants that take up contaminants from soil should lower the soil's contaminant concentrations to acceptable levels, provided the harvested amounts of contaminants exceed further inputs. Each cropping would remove contaminants from the area. The metal-rich biomass would be burned, fermented or used in gasification to reduce its volume. Residual material that is rich in

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Phytoextraction requires that plants accumulate large amounts of contaminants into the above-ground biomass. Hyperaccumulator plants do have this property (Brooks et al., 1977), but most of them produce little biomass so that their extraction efficiency is usually limited. In addition, agronomic cultivation techniques would still need to be developed for these plants. As an alternative strategy it has been proposed to use high biomass crop plants for which agricultural techniques exist and increase the accumulation of the contaminating metals by the application of amendments that increase their bioavailability. The addition of chelants such as EDTA can dramatically increase plant metal uptake (Huang and Cunningham, 1996; Blaylock et al., 1997). However, the formation of soluble metal-organic chelates also increases the risk of metal leaching to groundwater, in particular if the chelant is resistant to biodegradation (Nowack et al., 2006). The targeted application (e.g. the injection into the root zone) of low amounts of biodegradable compounds such as NTA or EDDS (Kulli et al., 1999; Tandy et al., 2006) may reduce risks to acceptable levels. Another possibility to solubilise metals such as Cd and Zn in soil is artificial soil acidification. In practice this can be achieved by the addition of elemental sulphur (S<sub>8</sub>). The oxidation of elemental sulphur to sulphuric acid, which is catalyzed spontaneously through the activity of autochthonous bacteria such as Thiobacillus,

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generates acidity, resulting in a decrease in soil pH (Nor and Tabatabai, 1977). Decreasing soil pH increases the solubility of metal cations and thus also their bioavailability for plant uptake. The potential to enhance plant metal uptake by application of elemental sulphur or biodegradable chelants has been demonstrated in pot experiments (Tichý et al., 1997; Wenger et al., 2002; Meers et al., 2005), but there is little information on how these techniques work under field conditions. Kayser et al. (2000) applied NTA and elemental sulphur for 1 year on a metalcontaminated agricultural field and found that even with artificially enhanced solubilisation of the contaminants, phytoextraction was still by far too slow to be viable in practice, unless the land could be used at the same time to produce an income that makes the whole operation profitable. The combination of phytoremediation and crop production is known as phytomanagement (Robinson et al., 2007; Domínguez et al., 2008). Potential plant products are non-food products such as biofuel, fibre, wood or, depending on the contamination level, animal feed. In order to show the viability of this approach, field experiments are necessary to test the implementation in agronomic practice. Long-term field trials are also necessary to test the effectiveness over time as climate and soil conditions vary from year to year and crop rotation is required. Therefore, the objective of this study was to investigate the potential use of sunflower, maize and tobacco in combination with the application of low amounts of biodegradable soil conditioners, such as NTA and sulphur, to remediate a heavy metal-contaminated soil and contemporaneously produce biomass that could be used as biofuel, animal feed or other purposes, depending on the levels of contaminant uptake.

# 2. Materials and methods

#### 2.1. Site description

The experiment was performed on an agricultural field on a eutric anthropic regosol (FAO soil order) at Witzwil, in the Bernese Seeland, Switzerland. The Seeland area ( $46^{\circ}58'60N$  and  $7^{\circ}2'60E$ , 432 m a.s.l.) has a temperate climate, with a mean annual temperature of 9 °C, and an average annual rainfall of 980 mm. It was a fen before being drained some 150 years ago and put into agricultural cultivation. To improve the soil fertility, municipal waste, including ash from coal-fired power plants, from the city of

Bern was applied between 1913 and 1954 on large parts of the area. Initially, the applied waste was mainly composed of organic matter (manure, municipal refuse and green waste). However, the fraction of indecomposable materials (waste materials containing plastic, glass or metal) increased until 1954 when waste application was discontinued. As a result of these applications, the soil became contaminated with heavy metals. The Bernese soil protection agency (Rvtz, 2001) reported Cd. Cu and Zn concentrations in excess of the guide (Cd. Zn) or trigger (Cu) values of the Swiss Federal "Ordinance Relating to Impacts on the Soil" (OIS, 1998), indicating that this site may negatively affect ecosystem quality or human health. The guide values for total Zn and Cd concentrations in the soil are 150 and 0.8 mg kg<sup>-1</sup>, respectively, and the trigger value for Cu is 150 mg kg<sup>-1</sup>. Analysis of white clover (Trifolium repens L.) and perennial ryegrass (Lolium perenne L.) samples, however, showed that contaminant uptake by these plants was minimal, indicating that the bioavailability of these contaminants may be low (Rytz, 2001). Based on the report by Rytz (2001) Cd, Cu and Zn were selected as problem contaminants to be further investigated.

#### 2.2. Experimental design

Maize (*Zea mays* L, cv. Magister), sunflower (*Helianthus annuus* L, cv. Sanluca), and tobacco (*Nicotiana tabacum* L, cv. Burley 92) were grown from 2000 to 2005 in a 3-year rotation scheme on three blocks in the experimental field. The rotation followed the order maize, sunflower, tobacco. All three crops were grown in each experimental year, but never consecutively on the same block (Fig. 1). Such rotation is standard agronomic practice in Switzerland and elsewhere. Maize and sunflower were sown around the 10th of May.

Each block was subdivided into 16 plots with a size of 12 m  $\times$  3 m each. Four treatments were applied in four replications to the plots of each block: (1) no amendment (control), (2) application of ammonium sulphate, (3) application of elemental sulphur and (4) application of nitrilotriacetic acid (NTA) (Fig. 1). The average amounts of sulphur applied in the ammonium sulphate treatment were 124, 195 and 69 kg ha<sup>-1</sup> yr<sup>-1</sup> for maize, tobacco and sunflower, respectively. In the elemental sulphur treatment, we applied 2139 kg ha<sup>-1</sup> yr<sup>-1</sup> sulphur with particle size



**Fig. 1.** Experimental design. Each block was cultivated as a separate field. The crops grown on each block changed from year to year in the same rotation but with a phase shift between the three blocks. Treatments started in 2000. The same treatments, C = control, SF = sulphur fertilizer (ammonium sulphate), S = sulphur (elemental sulphur), and NTA = nitrilotriacetic acid, were repeated on the same plots year.

Table 1			
Total applied	fertilizer	in	kg ha <sup>-1</sup>

Year	Maize	Maize		Tobacco		Sunflower	
	Block	N–P–K–Mg–S	Block	N–P–K–Mg–S	Block	N-P-K-Mg-S	
2000	А	110-40-190-38-120	В	170-20-200-40-127	С	90-26-330-66-209	
2001	В	110-40-190-38-120	С	170-20-200-40-127	А	90-26-330-66-209	
2002	С	110-40-190-38-120	А	170-20-200-40-127	В	90-26-330-66-209	
2003	А	80-40-190-38-120	В	170-20-200-40-127	С	90-26-330-66-209	
2004	В	100-50-200-40-127	С	170-20-215-43-136	А	90-26-330-66-209	
2005	С	145-50-200-40-127	А	170-20-215-43-136	В	90-26-330-66-209	

N-P-K-Mg-S: N as NH<sub>4</sub>NO<sub>3</sub> or (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, P as Super Triple Phosphate (Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>·H<sub>2</sub>O), and K, Mg and S as Patentkali (K<sub>2</sub>SO<sub>4</sub> and MgSO<sub>4</sub>).

of 0.5-3.0 µm to each crop. Elemental sulphur and ammonium sulphate were applied just before sowing. In the NTA treatment, 110 ml of 200 mM NTA was injected into a depth of 20 cm by a selfmade syringe with four holes (pointing in the four directions) on both sides of each plant at a distance of 15 cm to the stems, corresponding to an application rate of 82.8 kg NTA ha<sup>-1</sup> yr<sup>-1</sup>. NTA was applied 4 to 6 weeks after sowing, when the plant height was 30-40 cm. The rates of S and NTA applications were chosen on the basis of previous green house experiments (Wenger et al., 2002). The same treatments were repeated on the same plots annually for the duration of the experiment. All blocks were fertilized (N, P, K, Mg and S) according to the fertilizer recommendations of the Swiss Agricultural Research Stations (FAL and RAC, 2001). Fertilization was applied directly after sowing; N was applied a second time 2-3 weeks and a third time 4-5 weeks later (Table 1). Ammonium sulphate replaced ammonium nitrate as N source in the ammonium sulphate treatment. The total rates of N applied were the same in all treatments, but differed between the crops.

#### 2.3. Soil sampling and analysis

Before the beginning of the experiment (1999) and after each harvest, 16 soil cores (0-20 cm depth) were sampled on each plot, bulked to one composite sample, dried in a convection oven at 40 °C to constant weight and sieved to 2 mm. According to Swiss standard methods, we used an extraction with boiling 2 M HNO<sub>3</sub> to determine pseudo-total soil metal concentrations, while the soluble fractions of Cu, Zn and Cd were estimated using a 0.1 M NaNO<sub>3</sub> extractant (ART and ACW, 2007). Zinc and Cu were analysed using ICP OES (Inductively Coupled Plasma Optical Emission Spectroscopy), S by XRF (X-Ray Fluorescence Spectroscopy) and Cd by GF-AAS (Graphite Furnace Atomic Absorption Spectroscopy). The CaCO<sub>3</sub> content was determined using a calcimeter (ART and ACW, 2007) and organic carbon using the potassium dichromate oxidation method (ART and ACW, 2007). Soil pH was measured in H<sub>2</sub>O at a soil:solution ratio of 1:2.5. Soluble (plant available) P and K were extracted with CO2saturated water and soluble Mg with 0.0125 M CaCl<sub>2</sub> (FAL and RAC, 2001). Total and soluble metal concentrations were determined for the soil samples taken 1999, 2000, 2003 and 2005, soluble P, K and Mg for the samples taken in the years 1999, 2000, 2003 and 2004, while CaCO $_3$  and C $_{\rm org}$  were determined only for the samples taken 1999, 2000 and 2005. Soil pH was determined from 1999 to 2005.

In spring 2006, three plots per treatment were randomly selected for subsoil sampling. On each of the chosen plots, three soil cores (5 cm diameter) were taken down to a depth of 75 cm at increments of 25 cm using a hollow cylinder coring device (type HUMAX, Martin Burch AG, Luzern, Switzerland). The three sampling depths are designated as topsoil (0–25 cm), upper subsoil (25–50 cm), and lower subsoil (50–75 cm) in the following. The soil samples were oven-dried at 40 °C for 5 days and sieved to 2 mm mesh size. Soil pH, concentrations of total and soluble heavy metals, and contents of S, CaCO<sub>3</sub> and C<sub>org</sub> were determined.

#### 2.4. Plant sampling and analysis

Sunflowers were harvested always in the second half of August, maize and tobacco 1 month later. To avoid edge effects, plants of the outer rows and of the first and last 2 m of each plot were not included in the sampling. All the other plants were cut about 15 cm above ground and the total above-ground biomass (stem, leaves, seeds) was immediately passed through a chaff cutter. Roots were not collected. A 2 kg sample was taken at random from the harvest of each plot, oven-dried at 105 °C and ground to 1 mm, using an impact mill (AMA 102, Amman Langenthal, Switzerland). For P, K, Ca, Mg, Zn and Cu analysis, subsamples of 2.5 g plant material were ashed in a muffle furnace (prepASH 129, Precisa Instruments, Dietikon, Switzerland) at 600 °C for 2.5 h, dissolved in 5 ml of 6 M HCl, diluted to 50 ml with Millipore water, filtered and analysed using ICP OES. For Cd analysis subsamples of 0.5 g oven-dried plant material were microwave-digested in a mixture of 5 ml of HNO<sub>3</sub> (65%), 3 ml of  $H_2O_2$  (30%), and 2 ml of  $H_2O$ . The digested samples were diluted to 25 ml with Millipore water, filtered and analysed with GF-AAS. For N analysis, the Dumas combustion method was used with an elementar analyser (varioMAX CN-Analyser, Elementar Analysensysteme GmbH, Hanau, Germany), using 1 g subsamples of oven-dried plant material.

#### 2.5. Analytical quality assessment

For quality assurance, we analysed ISE (International Soilanalytical Exchange, Wageningen, The Netherlands) and IAG (International Analytical Group, Linz, Austria) inter-laboratory comparison soil and plant samples together with the experimental samples. The maximal relative standard deviation (coefficient of variation) of repeated measurements of the reference samples was 7% and the respective maximal relative bias was 5% for all elements.

### 2.6. Statistical analysis

Treatment effects were determined using analysis of variance in combination with post hoc analysis by Bonferroni tests. We used the following model (1) with the factors plant and treatment and two normally distributed and mutually independent random effects of block and year:

$$y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \delta_{l(k)} + \varepsilon_{ijklm}, \tag{1}$$

where  $y_{ijklm}$  is the response (i.e. dependent) variable,  $\mu$  is the mean response,  $\alpha_i$  is the effect of treatment i(1 = control; 2 = ammoniumsulphate; 3 = elemental sulphur; 4 = NTA),  $\beta_j$  is the effect of plant species  $j(1 = \text{sunflower}; 2 = \text{maize}; 3 = \text{tobacco}), (\alpha\beta)_{ij}$  represents the interaction between treatment i and plant j,  $\gamma_k$  is the random effect of the year  $k(1 = 2000, ..., 6 = 2005), \delta_{l(k)}$  is the random effect of the block l(1 = block A, 2 = block B, 3 = block C) in year k, and  $\varepsilon_{ijklm}$  is a normally distributed independent error (m = 1, ..., 4). Each metal was analysed separately. Differences with p < 0.05

7.8

were considered significant. Data were log-transformed to get homoscedastic residuals. As we found significant interaction effects between treatments and plants, we analysed the treatment effects on metal accumulation also separately for each plant.

#### 3. Results

# 3.1. Soil

On the basis of the topsoil (0-20 cm depth) samples taken at the beginning of the experiment (1999), which on average had sand, silt and clay fractions of 0.57, 0.24, and 0.19, respectively, the soil texture was classified as a sandy loam according to the US Soil Taxonomy. The pH of the soil was 7.4, the CaCO<sub>3</sub> content 3.75%, and the Corg content 12.2%. The mean total concentrations of Cd, Cu and Zn in the 1999 samples were  $1.37\pm0.03,\ 536\pm14,\ and$  $684 \pm 16 \text{ mg kg}^{-1}$ , respectively. Table 2 gives the properties of the untreated soil for the three sampling depths, topsoil (0-25 cm), upper subsoil (25-50 cm) and lower subsoil (50-75 cm), measured in 2006. The properties of the topsoil agree well with those of the composite samples (0-20 cm) taken in 1999 (see above), with the exception that the soil in the plots chosen for sampling in 2006 had a higher clay content (30.5%) than the average of the experimental field, indicating local variations in soil texture. The sand content decreased from 41% in the topsoil to 3% in the lower subsoil. Due to the increased silt content, the texture of the lower subsoil was classified as silty clay loam. Soil pH and CaCO<sub>3</sub> content were highest in the topsoil, whereas Corg and S content were highest in the lower subsoil. The high CaCO<sub>3</sub> content of the topsoil can be explained with the input of ashes that were deposited with the city wastes in the early 20th century, while the high Corg content of the lower parts of the profile is due to incomplete decomposition and residues of former peat layers. The contents of plant available nutrients in the topsoil were classified as "moderate" for P, "adequate" for Mg, and "available in reserve" for K according to Swiss standards (FAL and RAC, 2001). Total Cd, Cu and Zn concentrations were highest in the topsoil, still considerably above typical Swiss background concentrations in the upper subsoil and in the range of typical background concentrations in the lower subsoil. In addition, the soluble soil Cd. Cu and Zn concentrations decreased with depth. In contrast to Cu, this drop was not proportional to the decrease in total metal concentrations for Cd and Zn. The soluble Cd concentration decreased by less than a factor of 2 with depth from 1.3 in the topsoil to 0.8  $\mu$ g kg<sup>-1</sup> in the lower subsoil, while the total Cd concentration decreased by more than a factor of 5. The soluble Zn concentration decreased 4-fold from the topsoil to the subsoil, but did not decrease further with depth in the subsoil although the total Zn concentration decreased by a factor of almost 10 from the upper to the lower subsoil. The higher ratios between soluble and total Cd and

7.6 7.4 7.2 Ч 7.0 ∧ control ammonium sulphate 68 elemental sulphur NTA 6.6 2005 1999 2000 2001 2002 2003 2004

**Fig. 2.** Topsoil pH (0–20 cm) in the four treatments between 1999 and 2005. The error bars show the standard error of the means.

Zn in the subsoil than in the topsoil correspond well to the drop in pH and  $CaCO_3$  content from topsoil to subsoil. The depth profiles thus do not indicate that any substantial leaching of these metals occurred.

Significant treatment effects were limited to the topsoil of the elemental sulphur treatment, where the topsoil pH decreased from 7.4 to 6.7 between 1999 and 2002, and remained at this level for the rest of the experiment (Fig. 2). The soil CaCO<sub>3</sub> content decreased over the experimental period in all treatments. The decrease was greater in the sulphur treatment than in the ammonium sulphate and the NTA treatments, which did not differ from the control (Fig. 3). Conversely, soluble Mg and Cd increased over time. The sulphur treatment caused a sharp increase in soluble Zn (Fig. 3) and a greater increase in soluble Mg than in the other treatments. There were no significant treatment effects at lower depths and no effects on the NaNO<sub>3</sub>-soluble Cu concentration, the total soil Cd, Zn and Cu concentrations or the organic carbon content.

### 3.2. Plants

None of the plants showed deficiency symptoms, and yields were within the usual range for these three crops in Switzerland (Walter et al., 2001) for all treatments. Compared to the concentration ranges considered to indicate sufficient supply according to Bergmann (1993), the concentrations of N, P, K and Mg were suboptimal in maize, while N was suboptimal in sunflower (Table 3). Furthermore, N and Ca were above and Mg below the respective ranges of mineral sufficiency for tobacco. Only the sulphur treatment had a significant effect on the accumulation of

Table 2

Properties (mean values and standard errors in parenthesis) of the untreated soil, sampled at three depths (0-25, 25-50, and 50-75 cm) in spring 2006.

Soil properties	Topsoil (0–25 cm)	Upper subsoil (25–50 cm)	Lower subsoil (50–75 cm)
рН	7.53 (0.02)	7.23 (0.04)	6.94 (0.03)
CaCO <sub>3</sub> (%)	3.78 (0.48)	2.16 (0.62)	0.11 (0.03)
C <sub>org</sub> (%)	10.03 (0.26)	12.92 (0.79)	18.86 (0.73)
$CEC_{pot}$ (cmol <sup>+</sup> kg <sup>-1</sup> )	79.62 (1.57)	127.05 (11.55)	148.09 (9.71)
Clay (%)	30.53 (3.06)	49.15 (6.74)	38.68 (9.33)
Silt (%)	28.35 (1.17)	27.42 (0.68)	58.28 (10.00)
Sand (%)	41.12 (4.03)	23.43 (6.13)	3.03 (0.97)
$Cd_{total} (mg kg^{-1})$	1.35 (0.03)	0.69 (0.09)	0.23 (0.05)
$Cu_{total}$ (mg kg <sup>-1</sup> )	477 (19)	244 (37)	28 (9)
$Zn_{total}$ (mg kg <sup>-1</sup> )	652 (31)	381 (67)	39 (13)
S <sub>total</sub> (mg kg <sup>-1</sup> )	171 (7)	256 (24)	582 (29)
$Cd_{soluble}$ (µg kg <sup>-1</sup> )	1.32 (0.08)	0.90 (0.07)	0.79 (0.4)
$Cu_{soluble}$ (µg kg <sup>-1</sup> )	469 (28)	282 (39)	28 (9)
$Zn_{soluble} (\mu g k g^{-1})$	81.8 (4.4)	27.5 (9.3)	21.1 (7.2)



**Fig. 3.** CaCO<sub>3</sub> content and soluble Mg, Zn and Cd concentrations of the topsoil (0-20 cm) samples taken in different experimental years as indicated in the graphs. Different letters indicate significant differences (p < 0.05) between the treatments of the respective year. Error bars show the standard errors of the means.

nutrients. It increased the P concentration in maize from 1.85 to  $2.15 \text{ g kg}^{-1}$  and the Mg concentration in tobacco from 3.05 to  $3.33 \text{ g kg}^{-1}$  (data not shown), thus bringing the concentration of these two nutrients closer to the optimal range. The absence of deficiency symptoms despite the indication of suboptimal nutrient concentrations according to the reference values given by Bergmann (1993) may indicate that these reference values do not apply to the cultivars used in our study. Bergmann (1993) mentions that variability between cultivars is common. Plant nutrient concentrations can also vary substantially with weather conditions before harvest and with the age of the plant at harvest time (Bergmann, 1993).

The increase in NaNO<sub>3</sub>-extractable soil Cd and Zn over the years in the sulphur-amended plots coincided with a significant increase in Cd and Zn accumulation in the tobacco shoots (Fig. 4). In maize and sunflower, only Zn but not Cd concentrations increased. The application of NTA slightly increased the Zn concentration in maize, although we observed no effect on soluble Zn in the soil. None of the treatments affected the concentration of Cu in any of the three experimental crop plants, which agrees well with the corresponding lack of effect on soluble soil Cu concentration. Compared to the controls, the sulphur treatment increased the mean Zn-to-Cd ratio in maize and sunflower by around 30% and 10%, respectively. In tobacco, the Zn-to-Cd ratio remained unaffected.

Fig. 5 shows that the variations in yields and plant metal concentrations between years were generally larger than the differences between treatments in the same year. The variations in

yield and heavy metal accumulation were not correlated to the monthly means of temperature, precipitation or evapotranspiration, at nearby weather stations (Table 4). Fig. 6 shows that the effect of the sulphur treatment increased over the years in the case of Zn and Cd uptake by tobacco. The Zn concentration in maize increased, if we consider the value of 2001 to be an outlier. In sunflower, however, the effect of S on Zn uptake showed no consistent trend over the years. The increased accumulation of Zn and Cd by S-treated tobacco corresponds well with the continuous decrease in soil pH over the first three experimental years in the sulphur treatment.

Given that the variations in growth and plant metal accumulation from year to year did not exhibit any close correlation, the annual extraction of metals from the soil was not constant over time. The Cu extraction rates averaged 165, 270 and 285 g ha<sup>-1</sup> yr<sup>-1</sup>, for maize, sunflower and tobacco, respectively, over the 6 years of the experiment. The treatment effects on metal extraction rates corresponded to those on metal accumulation, as there was no significant treatment effect on yields. The sulphur treatment increased the Zn extraction by maize, sunflower and tobacco by 34%, 45% and 22% (Table 5) and the Cd extraction by 0%, 34% and 36% (Table 6) for the respective plants. The highest Zn extraction was obtained by the sulphur-treated sunflowers in 2004 (best-case scenario), with an extraction rate of >2 kg ha<sup>-1</sup>, which was more than twice the average rate of the untreated sunflowers (control). and more than three times the average Zn extraction rate of tobacco, the weakest Zn extractor of the three crops. For Cd, tobacco was the best extractor. The highest Cd extraction rate (10.5 g  $ha^{-1}$ ) occurred

#### Table 3

Mean concentrations of macronutrients in the plants of the control treatment (averages for the years 2000–2005, standard errors in parenthesis), in comparison to the concentration ranges considered to indicate sufficiency according to Bergmann (1993).

Nutrient (gkg <sup>-1</sup> )	Maize		Sunflower		Tobacco	Tobacco	
	Measured	Reference	Measured	Reference	Measured	Reference	
Ν	15.39 (0.16)	35–50	22.78 (1.16)	30–50	29.95 (0.45)	22-25	
Р	1.85 (0.04)	3.5-6.0	3.05 (0.21)	2.5-5.0	2.49 (0.14)	2.5-4.5	
К	10.95 (0.29)	30-45	39.29 (2.48)	30-45	36.61 (1.94)	25-45	
Ca	3.43 (0.09)	3-10	20.43 (1.08)	8-20	27.93 (1.57)	13-24	
Mg	1.45 (0.04)	2.5-5.0	4.13 (0.30)	3–8	3.05 (0.16)	4-8	



**Fig. 4.** Mean concentrations of Zn, Cd and Cu in the above-ground parts of maize, sunflower and tobacco harvested between 2000 and 2005. Different letters indicate significant differences (p < 0.05) between the treatments. Error bars indicate the standard errors of the means.



Fig. 5. Treatment effects on the annual yield (bar plots with standard errors of the means) and heavy metal concentrations (symbols: Zn 5. Cu 5. Cd 5.

#### Table 4

Climate data for the growing seasons (May-September) of the experimental years 2000-2005 recorded at weather stations close to the experimental site.

Parameter	Month	2000	2001	2002	2003	2004	2005
Temperature (°C)	May	15	16	13	15	13	14
	June	18	16	19	23	17	19
	July	17	19	19	21	19	19
	August	19	20	18	23	19	17
	September	16	12	14	15	16	16
Precipitation (mm)	May	94	48	127	61	52	66
	June	66	133	79	63	85	44
	July	155	112	116	73	75	39
	August	114	100	145	118	194	90
	September	56	119	57	65	28	78
ET <sub>pot</sub> (mm)	May	119	120	89	106	119	105
	June	148	125	143	179	126	156
	July	127	141	135	162	138	150
	August	127	131	109	162	119	114
	September	96	68	72	98	91	83

Temperatures and  $ET_{pot}$  values are averages of the weather stations Neuenburg ( $47^{\circ}0'1''N$ ,  $6^{\circ}57'14''E$ , 484 m a.s.l.) and Payerne ( $46^{\circ}48'49''N$ ,  $6^{\circ}56'33''E$ , 490 m a.s.l.). The potential evapotransportaion ( $ET_{pot}$ ) was calculated from data of these two stations, using the FAO Penman–Monteith equation (Allen et al., 1998). The precipitation was recorded at the weather station of Witzwil.

in 2003 with the sulphur-treated tobacco. This was twice the average Cd extraction rate of untreated tobacco, and nine times the average extraction rate of maize, the weakest Cd extractor.

# 4. Discussion

# 4.1. Soil properties

Whereas the total concentrations of Zn, Cd and Cu exceeded the respective guide or trigger values of the Swiss Federal "Ordinance Relating to Impacts on the Soil" (OIS, 1998), the NaNO<sub>3</sub>-soluble concentrations were below critical values which are 0.5 mg Zn, 0.02 mg Cd and 0.7 mg Cu per kg soil. The low metal solubility can be attributed primarily to the high pH and organic matter content of the soil. The contamination had aged for decades in the Witzwil soil (50–100 years), which may have resulted in reduced solubility of the contaminating metals over time (Tuin and Tels, 1990).

The experimental soil had an organic carbon content of 10–12% in the topsoil. Soil organic matter is an important sorbent for heavy

metals, especially at a soil pH above 6.5 (Alloway and Jackson, 1991). All three contaminants are strongly bound by soil organic matter, in particular Cu (Sposito, 2008). The decrease in soil pH in the sulphur treatment was insufficient to significantly increase Cu solubility. This would have required a pH decrease below 5 (Hornburg and Brümmer, 1993). In contrast, Cd and Zn solubility start to increase strongly with increasing soil acidification already at pH values below 7 and 6, respectively (Hornburg and Brümmer, 1993). After 6 years of sulphur application, the pH of the experimental topsoil (0-20 cm) was still close to neutral (pH 6.7). The high buffering capacity of the  $CaCO_3$  in the soil reduced the acidifying and metal mobilizing effects of the S. Given that the carbonate content decreased to less than half of the initial concentration during the experiment, a stronger decrease in soil pH would have required another 6 years of sulphur applications at our annual dosage of 2.14 t ha<sup>-1</sup>.

In contrast to the sulphur treatment, we observed no increase in metal solubility in the NTA treatment, not even for Cu, which is known to form strong complexes with amino-carboxylates



Fig. 6. Effect of the sulphur treatment (2000–2005) on the Cd and Zn concentrations of tobacco and on the Zn concentration of maize and sunflower over time. The symbols represent the mean difference ( $\Delta$ ) of the Cd or Zn concentration between control and sulphur treatment in the given year. The bars represent the standard errors of the means.

Table 5								
Zinc extraction (g ha-	<sup>1</sup> yr <sup>-</sup>	1) from soil by	maize,	sunflower	and tobacco,	, in control	and sulphur	treatment.

Maize			Sunfl	ower	Tobacco		
Year	Control	Sulphur	Control	Sulphur	Control	Sulphur	
2000	994 (58)	1015 (51)	531 (56)	577 (42)	378 (27)	382 (42)	
2001	989 (77)	1839 (107)	836 (38)	1360 (116)	932 (45)	924 (49)	
2002	1127 (72)	1309 (35)	790 (24)	1138 (50)	596 (35)	891 (6)	
2003	1165 (4)	1670 (74)	845 (51)	1087 (73)	624 (52)	743 (24)	
2004	961 (35)	1207 (47)	1215 (66)	2006 (150)	535 (9)	732 (36)	
2005	970 (78)	1308 (44)	767 (13)	1049 (34)	595 (19)	791 (46)	
Mean	1034 (33)	1392 (114)	831 (82)	1203 (175)	610 (67)	744 (72)	

Standard errors in parentheses. Framed: best-case scenario.

such as NTA (Nowack et al., 2006). Mobilization of Cu at higher soil pH could be achieved by the formation of Cu-complexes with dissolved organic ligands. The applied dose may have been too low for the NTA to compete with the undissolved organic matter of the soil for Cu. Furthermore, the applied NTA may have degraded too rapidly to result in a measurable effect on metal mobilization in the soil when it was sampled at harvest time. Tiedje and Mason (1974) reported that up to 80% of soil applied NTA had been degraded 24 days after application. They reported that NTA degradation rates in topsoils were positively correlated with the soil's organic matter content. The high organic matter content in the Witzwil soil thus might have accelerated NTA degradation. Wenger (2000) investigated the degradation kinetics of NTA by monitoring its effect on the NaNO3-extractable Zn concentration in a calcareous metal-polluted soil over time. The application of 5 mmol NTA kg<sup>-1</sup> dry soil increased the soluble soil Zn concentration from 4 to 200 mg kg<sup>-1</sup>, but after 20 days Zn solubility started to decrease again. With the addition of 25 mmol kg<sup>-1</sup> the mobilizing effect of NTA on soluble Zn disappeared within 63 days. The results reported by Wenger (2000) suggest that some mobilization may also have occurred in our NTA treatments, but may have been too ephemeral to be detectable still in the soil at harvest time and to significantly affect plant metal accumulation. Such a transient effect may, however, explain the small NTA effect on Zn accumulation by maize. A sustained NTA effect on soil metal solubility would require repeated applications during the growing season. However, this is impractical in larger field experiments and even less in agricultural practice.

#### 4.2. Plant concentrations

The relatively low plant metal concentrations may be partly due to short-range heterogeneity in the spatial distribution of the metals in the soil. Pronounced heterogeneity in the distribution of pollutants, even over short distances, is a typical phenomenon in field soils. Potted soils are usually homogeneous, thereby giving the roots little possibility to avoid contaminants. In contrast, under field conditions they generally have more possibility to evade contaminants by growing into zones of low concentrations (Keller, 2006). Contamination does not usually extend far into the subsoil. so that deeper roots encounter fewer toxicity problems than superficial roots. The contamination of the field soil studied here was more or less limited to the upper 50 cm of the soil. Roots of sunflower can reach depths of 1.5 m (Angelova et al., 2004), indicating that only part of the root system was involved in metal extraction. Keller et al. (2003) found a maximum rooting depth of 0.75 m for tobacco with a rapid decrease in rooting density below the first 0.1 m, whereas the rooting density of maize decreased less abruptly with depth, indicating that tobacco is more suitable for phytoextraction of contaminations in the upper layers and maize for phytoextraction of deeper contaminations. We found higher Cd and Cu concentrations in tobacco than in maize. The difference in Zn accumulation, however, was only marginal.

Plant metal concentrations and yield varied considerably from year to year. This shows that predictions of the time required to remediate a soil by means of phytoextraction are unreliable if based on experimental data from a single year only. Also Keller et al. (2003) found significant year-to-year variations in root development and metal uptake of different annual plants. To

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Cadmium extraction (g ha<sup>-1</sup> yr<sup>-1</sup>) from soil by maize, sunflower and tobacco, in control and sulphur treatment.

	Maize		Sunfle	ower	Tobacco		
Year	Control	Sulphur	Control	Sulphur	Control	Sulphur	
2000	1.16 (0.09)	1.13 (0.04)	1.84 (0.16)	2.25 (0.17)	6.24 (0.46)	6.53 (0.80)	
2001	1.14 (0.11)	1.05 (0.03)	1.27 (0.04)	1.79 (0.16)	5.87 (0.12)	6.36 (0.08)	
2002	1.38 (0.11)	1.66 (0.02)	3.40 (0.20)	4.79 (0.42)	3.15 (0.24)	5.72 (0.28)	
2003	0.97 (0.05)	1.01 (0.05)	2.53 (0.22)	3.51 (0.11)	7.26 (0.52)	10.52 (0.35)	
2004	0.94 (0.05)	1.08 (0.08)	3.53 (0.23)	4.43 (0.99)	5.91 (0.31)	8.63 (0.27)	
2005	1.37 (0.41)	0.97 (0.08)	2.67 (0.07)	3.65 (0.12)	6.55 (0.33)	9.96 (0.09)	
Mean	1.16 (0.07)	1.15 (0.10)	2.54 (0.33)	3.40 (0.44)	5.83 (0.53)	7.95 (0.76)	

Standard error in parentheses. Framed: best-case scenario.

calculate the remediation time ( $t_r$ ) needed to reach target heavy metal concentrations as given the guide values ( $c_{gv}$ ) of the Swiss Federal "Ordinance Relating to Impacts on the Soil" (OIS, 1998), more realistic estimates will be obtained with data from experiments with a duration of several years. Using the mean annual metal extraction ( $c_{me}$ ) over the 6 years of our experiment and assuming a linear decrease in soil metal concentration according to Kayser et al. (2000), beginning from the soil metal concentration in 1999 ( $c_0$ ), the calculated remediation time would be around 240, 2900 and 870 years for Cd, Cu, and Zn, respectively, using Eq. (2):

$$t_{\rm r} = \frac{c_0 - c_{\rm gv}}{c_{\rm me}}.\tag{2}$$

For Cd and Zn, these times would decrease by about a guarter were the soil pH maintained at 6.7. Using the value of the highest metal extraction rate achieved in our experiment, which was 2 kg ha<sup>-1</sup> for Zn in 2004 with sulphur-treated sunflower and 10.5 g ha<sup>-1</sup> for Cd in 2003 with sulphur-treated tobacco, then the needed remediation time would still be 360 years for Zn and 70 years for Cd. Even in this unrealistic "best-case" scenario, the time needed to reach the remediation target would still be unacceptably long. Nevertheless, the low metal accumulation rates can be advantageous, as this means that the biomass could be used for human or animal nutrition without unacceptable toxicity risks. The concentrations of Cd, Cu and Zn in our plants were in the same range as plants grown on uncontaminated land (Chaney, 1989), and well below concentrations shown to be harmful to animals (Underwood and Suttle, 1999). The Cd concentrations were also below the Swiss threshold value for undesired substances in animal fodder (FMBV, 1999), while for Zn and Cu no such threshold values exist. Sulphur application may even increase the fodder quality because of the increased Zn-to-Cd ratio. Zinc, in contrast to Cd, is an essential nutrient for plants, human and animals, and becomes toxic only at high concentrations. Zinc deficiency usually is a more serious problem in human and animal nutrition than Zn toxicity. In particular, Zn deficiency can promote the absorption and retention of Cd by human and animals (Tang et al., 1998; Underwood and Suttle, 1999). Thus increasing the ratio of Zn-to-Cd accumulation in food and fodder plants could be beneficial with respect to health risk reduction. Crop residues with elevated Zn and Cu concentrations could also be used as green manure on agricultural land where the concentrations of these two plant nutrients are low. Here, phytomanagement would not only allow a profitable agricultural use of the polluted soil, but also gradually reduce its contamination and in the same time ameliorate micronutrient-deficient soil elsewhere.

# 5. Conclusions

The uptake of Cd, Zn and Cu by the untreated plants was in the range of values found in crop plants grown on uncontaminated land. Therefore, the rate of extraction of these metals was low. The ammonium sulphate treatment had no effect on plant metal uptake. The NTA treatment was ineffective, except for a slight increase in Zn accumulation by maize. Elemental sulphur significantly increased the accumulation of Zn by all three experimental plants as well as the accumulation of Cd by tobacco. The applied elemental sulphur treatment would decrease the remediation time for the Zn and Cd contamination by about a quarter. It also improved the fodder quality of maize and sunflower by increasing the Zn-to-Cd ratio and by enhancing the nutritional status of tobacco (Mg) and maize (P). A higher rate of sulphur application would probably result in a faster and stronger decrease in soil pH and, correspondingly, in a higher Zn and Cd mobilization. However, the remediation time would still be unacceptably long. Cleansing the experimental soil by phytoextraction is thus unrealistic using the approach studied here. However, the results suggest that phytomanagement, i.e. to use the contaminated land profitably for the production of valuable biomass (animal feeding, biofortification, biofuel production and other economical purposes), would be a good strategy without running any substantial health risks for humans or animals.

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