

# Uptake and allocation of plant nutrients and Cd in maize, sunflower and tobacco growing on contaminated soil and the effect of soil conditioners under field conditions

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**Abstract** Contaminated land may in many cases still be used for agriculture, provided that crops are chosen appropriately, as the accumulation of contaminants varies greatly among cultivars and also plant parts. We aimed to determine whether maize (*Zea mays*), sunflower (*Helianthus annuus*) and tobacco (*Nicotiana tabacum*) grown on a heavy-metal contaminated soil containing copper (540 mg Cu kg<sup>-1</sup>), zinc (680 mg Zn kg<sup>-1</sup>) and cadmium (1.4 mg Cd kg<sup>-1</sup>) could be used to gradually remediate the soil, while producing valuable biomass. The soil was treated with either a normal fertiliser regime (control), elemental sulphur (S), or the biodegradable chelant NTA (nitrilotriacetic acid), to test how soil acidification or chelating organic compounds would affect the uptake and allocation of selected elements (Ca, Cd, Cu, Fe, K, Mg, Mn, P, S and Zn). The highest concentrations of Cd, Cu and Zn occurred in the leaves and/or roots, while seeds and grains contained much lower concentrations of these elements. All

these concentrations, however, were still in the ranges considered normal for the respective plant parts grown on uncontaminated soil. While sunflower and maize could be safely used as food and feed, tobacco would better be used for bioenergy than for cigarette production because of its relatively high foliar Cd concentration. The two treatments (S and NTA) had only slight effects on the uptake and allocation of plant nutrients and Cd. Thus, there was little benefit of these treatments for phytoextraction purposes at this site.

**Keywords** Heavy metal accumulation and distribution in crop plants · Phytomanagement · Tobacco · Maize · Sunflower · Zinc · Cadmium · Manganese · Copper

## Introduction

Heavy metal contamination of agricultural soils is a worldwide problem. In particular, the deposition of industrial and traffic emissions, excessive use of low-quality fertilizers, and the application of biowastes as fertilizers have substantially increased soil metal concentrations on large areas of agricultural land. Using contaminated land for the production of food or feed crop plants bears the risk that the contaminants are transferred into food and feed stuffs and ultimately create health risks for humans and animals. On the other hand, leaving low- or moderately-contaminated land fallow may be wasting a

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precious natural resource, given the increasing shortage of fertile agricultural soil.

Remediating such large areas of land may be prohibitively expensive or even impossible. Baker et al. (1994) and Chaney et al. (1997) suggested that using plants to extract contaminants from such soil (phytoextraction) may be a low-cost and gentle remediation option that restores the fertility of the soil. Unfortunately, phytoextraction is slow and may take centuries to reach regulatory clean-up standards, even for relatively mobile trace metals such as Cd or Zn (Kayser et al. 2000). However, the treatment time would be less important if the soil was used simultaneously to produce profitable crops, an approach also known as phytomanagement (Domínguez et al. 2008).

Phytomanagement aims to combine the production of valuable biomass, such as timber, bioenergy or stock fodder, with the gradual removal of the contaminants, and simultaneously mitigates risks by preventing the off-site movement of contaminants via leaching, runoff or erosion. Studies have shown that the accumulation of contaminants or nutrients by plants varies greatly among crops and cultivars and also differs between different parts of a plant (Kurz et al. 1999; Liu et al. 2007). Seeds and fruits generally accumulate metals at lower concentrations than leaves or roots (McLaughlin et al. 1996, 1999; Wenger et al. 2002a; Angelova et al. 2004; Liu et al. 2007). For example, maize seed produced on contaminated land may be suitable for animal feed (Wenger et al. 2002a), while the stems and leaves could be used for non-food purposes such as bioenergy production (Meher et al. 1995; Licht and Isebrands 2005; Amon et al. 2007).

We had tested such a phytomanagement approach in a 6-year field experiment that began in 2000 on a heavy-metal contaminated agricultural soil that had developed from a peat soil after artificial drainage in the late nineteenth century (Fässler et al. 2009). From 2000–2005, maize, sunflower, and tobacco were cultivated in this field experiment in a 3-year crop rotation scheme. Four treatments were applied: (1) elemental sulphur (S), (2) nitrilotriacetic acid (NTA), and (3) ammonium sulphate fertilizer ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) to increase soil metal availability for uptake by plants (Kulli et al. 1999; Kayser et al. 2000; Wenger et al. 2002b), and (4) control (no amendment).

As expected, the elemental sulphur applications decreased soil pH and CaCO<sub>3</sub> content, increased soil

Cd and Zn solubility, and enhanced the uptake of Zn by all three plant species as well as the uptake of Cd by tobacco. NTA only increased the accumulation of Zn in maize. No NTA effect was found in the soil. The (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> showed no effect on soil and plant metal uptake. None of the treatments affected crop yield. While the results indicated that soil cleansing via phytoextraction would require centuries, the plant concentrations were such that it may be viable use the plant material for fodder or bioenergy, while contemporaneously stabilising the soil and extracting some contaminants.

If the plants are to be used for food or stock fodder, then information on the metal allocation within the plants and how it is affected by potential amendments is critical, as allocation determines the potential human or animal exposure to contaminants. There is a lack of knowledge on the effects of S or chelant application on the distribution of metals in crop plants at harvest time. Studies on soil amendments focused on the soil-root and root-shoot transfer of contaminating metals, rather than on metal allocation in harvested plant parts.

There are few data on the impact of such soil treatments on the nutritional status of crop plants (Kayser 2000). Some studies dealing with S-applications investigated S-deficient plants. Nasreen and Huq (2002) showed that S-application to an S-deficient soil increased concentrations of N, P, K and S in sunflower to maximum values at a dose of 80 kg S ha<sup>-1</sup>, further additions caused a subsequent decrease in the plant concentrations of these macronutrients. Plant yield was positively correlated with plant macronutrient concentrations. Cimrin et al. (2008) reported similar results with lentils with an optimal dose of 120 kg S ha<sup>-1</sup>. However, neither of these studies investigated the effects of the treatment on soil pH. At a soil pH between 5.5 and 6.5 most nutrients are readily available for uptake by plants (Brady and Weil 2002). Changes in soil pH to values below or above this range may cause nutrient imbalances and reduce growth (Marschner 1995).

In this study, our objective was to (1) determine the allocation of the contaminating elements Cd, Cu and Zn, and several plant nutrients, in maize, tobacco and sunflower plants grown on the above mentioned field, (2) investigate how this allocation is affected by soil amendments with S and NTA and (3) evaluate the possibility of using the land for a profitable

production of these crops. For this purpose the field experiment described by Fässler et al. (2009) was extended for another year. The applied S and NTA dosages were doubled in comparison to the preceding years in order to produce stronger effects.

## Materials and methods

### Experimental site and design

The experiment was conducted on a field in the metal-contaminated area of Witzwil in the Bernese Seeland, Switzerland. The area is located between the lakes of Neuchâtel, Biel and Murten at an altitude of 432 m a.s.l. It has a mild and warm climate (9°C annual mean temperature) with an annual average rainfall of 980 mm. The sandy loam soil prevailing in this area had developed from a former peat soil that had been drained for agricultural use in the late nineteenth century. The soil contamination originated from the disposal of primarily organic city waste between 1913 and 1954, a practice originally also employed in order to increase the fertility of the reclaimed soil. While this objective was achieved, increasing amounts of contaminants including in particular Cd, Cu and Zn, were introduced into the soil in the same time, as the quality of the wastes decreased with time. The concentrations of these metals in the ploughed topsoil were approximately 1.4 mg kg<sup>-1</sup> for Cd, 540 mg kg<sup>-1</sup> for Cu, and 680 mg kg<sup>-1</sup> for Zn (Fässler et al. 2009).

Three blocks, each comprising 16 rectangular plot areas of 3 m × 12 m size were cultivated since 2000 in a 3-year crop rotation scheme of maize (cv. Magister), sunflower (cv. Sanluca), and tobacco (cv. Burley 92). The rotation was phase-shifted between the three blocks, so that all three crops were grown every year, but shifting to another field each year. All plots were fertilized according to the fertilizer recommendation of the Swiss Agricultural Research Stations (RAC and FAL 2001). Four treatments were applied in the years 2000–2005 in four replications on the sixteen plots within each block: (1) S, (2) NTA, (3) (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and (4) no amendment (control treatment). Each treatment was repeated on the same plot every year.

For the present study the experiment was extended one further year in 2006. For this purpose, S, NTA and control treatments were applied in spring 2006 on

the same plots as previously, but the rates of S and NTA application were doubled in comparison to the years before. Thus, in the S treatment 0.427 kg sulphur were applied per m<sup>2</sup>, resulting in a cumulative total addition of 1.71 kg m<sup>-2</sup> over the entire experimental time from 2000 to 2006. In the NTA treatment 110 ml of a 400 mM NTA solution (instead of 200 mM as in the years before) were injected on both sides of each plant at a distance of 15 cm from the stem. For reasons of feasibility, NTA was applied before the plants exceeded 40 cm in height, which was the case approximately 1.5 months after sowing.

### Sampling and sample analysis

Sunflower was harvested on the 15th of August, maize and tobacco on the 19th of September 2006. Three plants per treatment (S, NTA and control) and species were chosen at random for analysis. Roots were excavated and separated from the adhering soil by washing. Shoots were divided immediately into stems, leaves, seeds (maize and sunflower) or flowers (tobacco) and “remainder” (sunflower heads without kernels, maize cobs without grains and male flower of maize). Tobacco seeds were not fully developed at the time of sampling. Thus, it was not possible to separate them from the flowers. The samples were packed into plastic bags and immediately transported into the laboratory. Here, they were well washed with tap water, cut into pieces, using a chaff cutter (Wintersteiger, LH 120) for the tobacco stems and secateurs for the other material, and then oven-dried for 5 days at 60°C. The dried maize grains and tobacco flowers were ground to 0.5 mm using a centrifugal mill (Retsch, ZM1). The dried sunflower seeds were ground using a coffee grinder (Trisa 6200). All root samples as well as the tobacco and maize stems were pre-ground to 5 mm using a knife mill (Fuchs, 180S) and then ground to 0.75 mm using a heavy-duty cutting mill (Retsch, SM1). All other plant material was directly ground to 0.75 mm using the Retsch mill. Subsamples (0.5 g) were microwave-digested in 5 ml of HNO<sub>3</sub> (65%), 3 ml of H<sub>2</sub>O<sub>2</sub> (30%), and 2 ml of H<sub>2</sub>O. The digests were diluted to 25 ml with Millipore water and filtered.

Calcium, Cu, Fe, K, Mg, P, S and Zn were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES), Cd and Mn by ICP-MS (mass spectrometry). Certified material of Virginia

tobacco leaves (CTA-VTL-2) was digested and analyzed together with the samples for quality assurance. The maximal relative standard deviation of repeated measurements of the reference samples was 7% and the respective maximal relative bias was 5% for all elements.

After the plants had been harvested, 16 soil cores (0–20 cm depth) were sampled on each plot area, bulked to one composite sample, dried in an oven with air circulation at 40°C to constant weight, sieved to 2 mm and divided into subsamples for the following analyses. One 4-g subsample per sample was mixed with 0.9 g of wax and pressed into tablets under a pressure of 15 tons. The total element concentrations of Ca, Cd, Cu, Fe, K, Mg, Mn, P, S, and Zn were determined using X-ray fluorescence (XRF) spectrometer. A second subsample was digested using boiling 2 M HNO<sub>3</sub>, and the extract was analysed for the same elements as the first subsample except for S (ACW and ART 2007). The same elements as in the HNO<sub>3</sub> extract were also analysed in extracts obtained from a third series of subsamples using 0.5 M CH<sub>3</sub>COONH<sub>4</sub> and 0.03 M EDTA (ACW and ART 2007). The fourth subsample was extracted using 0.1 M NaNO<sub>3</sub> solution to determine soluble Cd, Cu and Zn concentrations (ACW and ART 2007).

Cadmium was analyzed by GF-AAS, the other elements by ICP-OES. Soil pH was measured in H<sub>2</sub>O at a soil:solution ratio of 1:2.5. The CaCO<sub>3</sub> content was determined with a calcimeter (ACW and ART 2007). Samples of certified sandy clay soil (no. 992, period 2005.2, obtained from the International Soil-analytical Exchange (ISE) program, Wageningen University) were analyzed together with the experimental samples for quality control. The maximal relative standard deviation of repeated measurements of the reference samples was 7% and the respective maximal relative bias was 5% for all elements.

#### Statistical data analysis

Treatment differences in the sample concentrations of the analyzed elements were determined by ANOVA and a post-hoc Bonferroni test, using SPSS (version 15). The data were log-transformed in order to normalize frequency distributions. Differences were judged significant if the error probability was 5% or less ( $P \leq 0.05$ ).

## Results and discussion

### Soil

The pH of the untreated topsoil was 7.7, the CaCO<sub>3</sub> content 2.7%, and the soil organic carbon (C<sub>org</sub>) content 10.7%. In 1999 the average soil pH had been 7.4, the CaCO<sub>3</sub> content 3.6%, and the soil organic carbon content 12.3% (Fässler et al. 2009). Our measurements here confirm the trends already observed in our previous study: a slight tendency of the soil pH to increase over the years, and a marked decrease in CaCO<sub>3</sub> and C<sub>org</sub> contents. According to the fertilisation recommendations of the Swiss Agricultural Research Stations (RAC and FAL (2001), the Witzwil soil was adequately supplied with K and P and had a good stock in Mg (data not shown).

The concentrations of HNO<sub>3</sub>-extractable Cd, Zn, and Cu (Table 1) exceeded the respective guide values (Cd: 0.8 mg kg<sup>-1</sup>, Cu: 40 mg kg<sup>-1</sup>, Zn: 150 mg kg<sup>-1</sup>) of the Swiss Federal Ordinance Relating to Impacts on the Soil (OIS 1998). These guide values indicate a pollution level at which the agricultural and ecological quality of a soil may be adversely affected in the long term according to Swiss environmental legislation (USG 1983). The HNO<sub>3</sub>-extractable Cu also exceeded the respective trigger value for this element (150 mg kg<sup>-1</sup>), indicating potential endangerment to exposed humans or animals (OIS 1998). The NaNO<sub>3</sub>-extractable (“soluble”) metal concentrations, however, were not found to exceed the respective OIS guide values (Cd: 0.02 mg kg<sup>-1</sup>, Cu: 0.7 mg kg<sup>-1</sup>, Zn: 0.5 mg kg<sup>-1</sup>). These results indicated that the availability of these metals for uptake by plants did not exceed legally tolerable limits.

As a result of the S treatment over seven consecutive years, the soil pH decreased by around one unit to a value of 6.5 at harvest time in 2006. The decrease in soil pH would have been greater had it not been buffered by the dissolution of CaCO<sub>3</sub>. In the S treatment the CaCO<sub>3</sub> content decreased from approximately 3.6% to 1.4% over the same 7 years. The high organic matter (OM) content added to the buffering capacity of the soil. Soil OM has its highest buffer effect at intermediate pH values (5–7) (Brady and Weil 2002).

The S treatment did not significantly change the total concentration of any of the analyzed soil

**Table 1** Concentrations of selected elements in the untreated soil

| Element | XRF ( $\text{g kg}^{-1}$ ) | $\text{HNO}_3$ extraction ( $\text{g kg}^{-1}$ ) | EDTA extraction ( $\text{g kg}^{-1}$ ) | $\text{NaNO}_3$ extraction ( $\text{mg kg}^{-1}$ ) |
|---------|----------------------------|--|--|--|
| Ca      | 37.63 (0.60)               | 33.15 (1.74)                                     | 16.65 (0.47)                           | nd   |
| Cd      | nd                         | 0.0014 (0.0001)                                  | 0.0013 (0.0000)                        | 0.0017 (0.0004)                                    |
| Cu      | 0.59 (0.03)                | 0.53 (0.04)                                      | 0.28 (0.01)                            | 0.381 (0.011)                                      |
| Fe      | 39.33 (0.84)               | 20.42 (1.11)                                     | 0.74 (0.02)                            | nd   |
| K       | 10.44 (0.10)               | 0.74 (0.06)                                      | 0.18 (0.01)                            | nd   |
| Mg      | 4.86 (0.07)                | 2.95 (0.14)                                      | 0.28 (0.01)                            | nd   |
| Mn      | 1.05 (0.02)                | 1.00 (0.05)                                      | 0.13 (0.00)                            | nd   |
| P       | 2.48 (0.06)                | 1.69 (0.09)                                      | 0.06 (0.00)                            | nd   |
| S       | 3.13 (0.08)                | nd   | nd                                     | nd   |
| Zn      | 0.80 (0.03)                | 0.68 (0.04)                                      | 0.14 (0.01)                            | 0.081 (0.014)                                      |

Standard errors of the means are in parentheses

XRF X-ray fluorescence, nd not determined

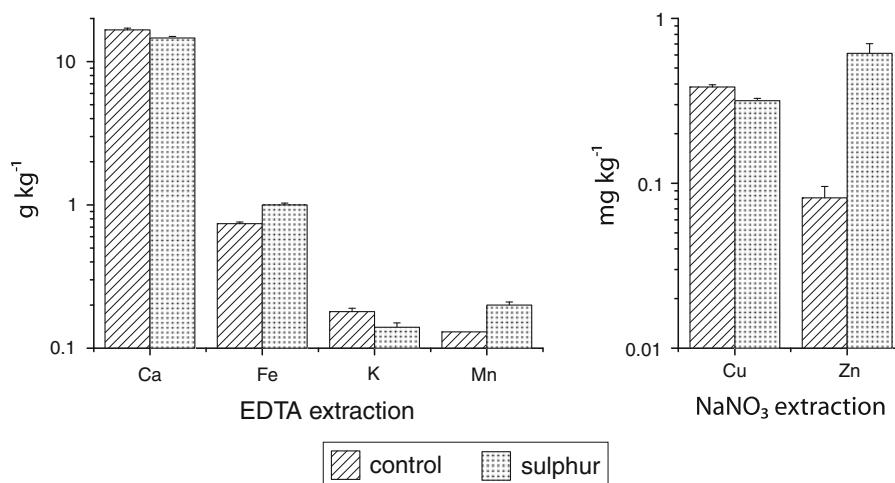
elements, except for the concentration of S itself, which was more than doubled (data not shown). However, it increased the EDTA-extractable Fe and Mn concentrations as well as the  $\text{NaNO}_3$ -extractable Zn concentration, whereas it decreased the  $\text{NaNO}_3$ -extractable Cu and the EDTA-extractable Ca and K concentrations (Fig. 1). The only significant effect of the NTA treatment was to increase EDTA-extractable P from 0.06 to 0.08  $\text{g kg}^{-1}$ .

#### Plant growth and element uptake on untreated soil

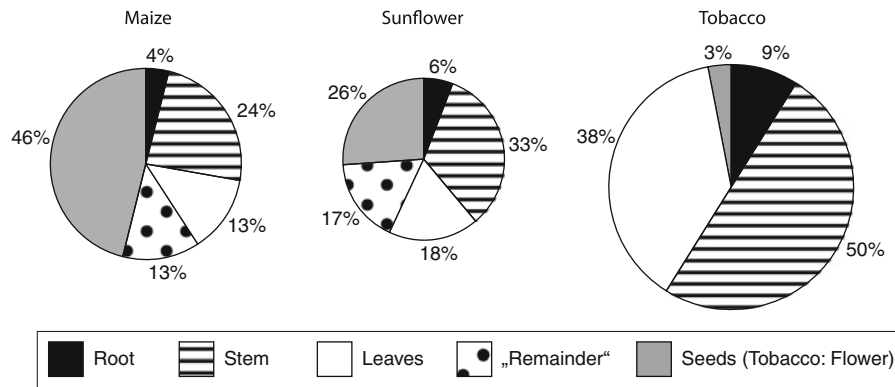
Plant growth appeared to be normal. There were no visible symptoms of deficiency or toxicity. The yield

was 20.5  $\text{t ha}^{-1}$  for maize, 9.2  $\text{t ha}^{-1}$  for sunflower, and 7.8  $\text{t ha}^{-1}$  for tobacco, which was in or above the normal range for these crops in Switzerland (Walter et al. 2001). The total aboveground biomass of single plants was  $0.25 \pm 0.02 \text{ kg}$ ,  $0.21 \pm 0.01 \text{ kg}$ , and  $0.32 \pm 0.05 \text{ kg}$  for maize, sunflower, and tobacco, respectively. Fig. 2 shows the biomass ratios between different parts of the three plant species. Maize grains amounted to 46%, sunflower kernels to 26% and tobacco flowers only to 3–4% of the total dry weight (including roots) of the respective plants.

Judged by the respective lower and upper threshold concentrations given by Bergmann (1993) to indicate the ranges of sufficient nutrient supply for different



**Fig. 1** The effect of S on soil metal solubility in EDTA extracts (left) and  $\text{NaNO}_3$  extracts (right). All depicted element concentrations were significantly changed by the sulphur treatment. Error bars represent the standard errors of the means



**Fig. 2** Dry weight proportions of maize (*left*), sunflower (*middle*) and tobacco (*right*) parts of the plants of the control treatment. “Remainder” consists of cobs (without grains) and male flowers in maize and heads without kernels in sunflower.

The areas of the circles are proportional to the total dry weights of the plants ( $0.25 \pm 0.05$  kg,  $0.21 \pm 0.01$  kg, and  $0.32 \pm 0.05$  kg for maize, sunflower and tobacco, respectively)

crop plant species, the maize plants should have been considered slightly deficient in Cu, Ca, Fe, Mg and P, and even more clearly deficient in K and Mn (Table 2). Potassium only reached 1/4 and Mn 1/8 of the lower sufficiency threshold. Potassium and Mn concentrations were also below the respective lower threshold values in sunflower. In tobacco, Mg, Mn and P concentrations were deficient, while Cu was slightly above the respective threshold values. All other elements were within the respective ranges of sufficiency.

That no deficiency symptoms were seen, even though the concentrations of some elements in each plant species were below the sufficiency threshold given by Bergmann (1993) for the respective species, indicates that not all of these values may apply to the cultivars used in the present study. Bergmann (1993) states that substantial variation between cultivars is common. Furthermore, the published sufficiency threshold values may not fully apply to the particular edaphic and climatic conditions of the study site.

**Table 2** Elemental concentrations of tobacco, sunflower and maize shoots grown on the untreated contaminated soil in comparison to reference values given by Bergmann (1993)

| Element | Unit                | Maize        |                      | Sunflower    |                      | Tobacco      |                      |
|---------|---------------------|--------------|----------------------|--------------|----------------------|--------------|----------------------|
|         |                     | Measured     | Reference            | Measured     | Reference            | Measured     | Reference            |
| Ca      | g kg <sup>-1</sup>  | 2.38 (0.04)  | 3–10 <sup>a</sup>    | 16.63 (0.10) | 8–20 <sup>a</sup>    | 22.80 (0.93) | 13–24 <sup>a</sup>   |
| Cd      | mg kg <sup>-1</sup> | 0.04 (0.00)  | 0.2–3.0 <sup>b</sup> | 0.28 (0.00)  | 0.2–3.0 <sup>b</sup> | 0.80 (0.02)  | 0.2–3.0 <sup>b</sup> |
| Cu      | mg kg <sup>-1</sup> | 3.16 (0.07)  | 7–15 <sup>a</sup>    | 18.21 (0.33) | 10–20 <sup>a</sup>   | 18.58 (0.72) | 8–15 <sup>a</sup>    |
| Fe      | mg kg <sup>-1</sup> | 42.00 (2.88) | 50–250 <sup>c</sup>  | 64.60 (8.07) | 50–250 <sup>c</sup>  | 76.20 (7.18) | 50–250 <sup>c</sup>  |
| K       | g kg <sup>-1</sup>  | 7.54 (0.37)  | 30–45 <sup>a</sup>   | 25.84 (2.10) | 30–45 <sup>a</sup>   | 27.38 (2.18) | 25–45 <sup>a</sup>   |
| Mg      | g kg <sup>-1</sup>  | 1.13 (0.05)  | 2.5–5.0 <sup>a</sup> | 3.38 (0.05)  | 3–8 <sup>a</sup>     | 2.67 (0.04)  | 4–8 <sup>a</sup>     |
| Mn      | mg kg <sup>-1</sup> | 4.80 (0.14)  | 40–100 <sup>a</sup>  | 22.80 (0.43) | 25–100 <sup>a</sup>  | 17.90 (0.81) | 50–120 <sup>a</sup>  |
| P       | g kg <sup>-1</sup>  | 1.51 (0.04)  | 3.5–6.0 <sup>a</sup> | 2.70 (0.20)  | 2.5–5.0 <sup>a</sup> | 2.01 (0.04)  | 2.5–4.5 <sup>a</sup> |
| S       | g kg <sup>-1</sup>  | 1.01 (0.02)  | 1–5 <sup>d</sup>     | 1.63 (0.02)  | 1–5 <sup>d</sup>     | 3.38 (0.09)  | 1–5 <sup>d</sup>     |
| Zn      | mg kg <sup>-1</sup> | 39.60 (1.50) | 30–70 <sup>a</sup>   | 61.40 (1.25) | 30–80 <sup>a</sup>   | 63.10 (1.63) | 25–70 <sup>a</sup>   |

Standard errors of the means are in parentheses

<sup>a</sup> Sufficient range of mineral nutrients in the specific crop plant (tobacco, sunflower or maize)

<sup>b</sup> Normal range of heavy metal concentration in plants, grown on uncontaminated soils

<sup>c</sup> Sufficient range of Fe concentration in plants

<sup>d</sup> Range of S concentration in plants, grown under normal conditions

Both, soil and climate can have substantial influence on nutrient uptake (Bergmann 1993).

Plant Cd concentrations did not exceed values typically found on uncontaminated land. Copper and Zn concentrations were within the respective sufficiency ranges, indicating sufficient supply of these elements as micronutrients according to Bergmann (1993), with the exception that Cu just slightly exceeded the upper threshold of sufficiency for this element in tobacco (Table 2). The low uptake of these metals despite of their high total concentrations in the soil can be attributed to the alkaline soil pH and the high OM content. Low bioavailability of all three metals is also indicated by the rather low  $\text{NaNO}_3$ -extractable metal concentrations as compared to the respective guide values.

#### Element allocation in plants on untreated soil

The highest concentrations of the macronutrients S, Ca, K, and Mg occurred in the leaves of the three investigated species (Fig. 3). In maize, the concentrations of these elements differed less among roots, stems and leaves than in tobacco and sunflower. In particular, there were no significant differences in S, Ca, K and Mg concentrations in the leaves and roots of maize. The concentration of Ca in the seeds of maize and sunflower was significantly lower than in the other parts of these two species. This may be attributed to the low phloem mobility of this element (Marschner 1995).

In contrast to the other nutrients, the highest P concentrations were found in the seeds of maize and sunflower and of tobacco flowers. In seeds, most phosphate is bound as phytate (Marschner 1995). In cereal grains, nuts and legume seeds, phytate-P may represent up to 80% of the total seed P. Phytate plays an important role in pathogen resistance of seedlings, which is a major factor for seedling survival and growth (Frossard et al. 2000).

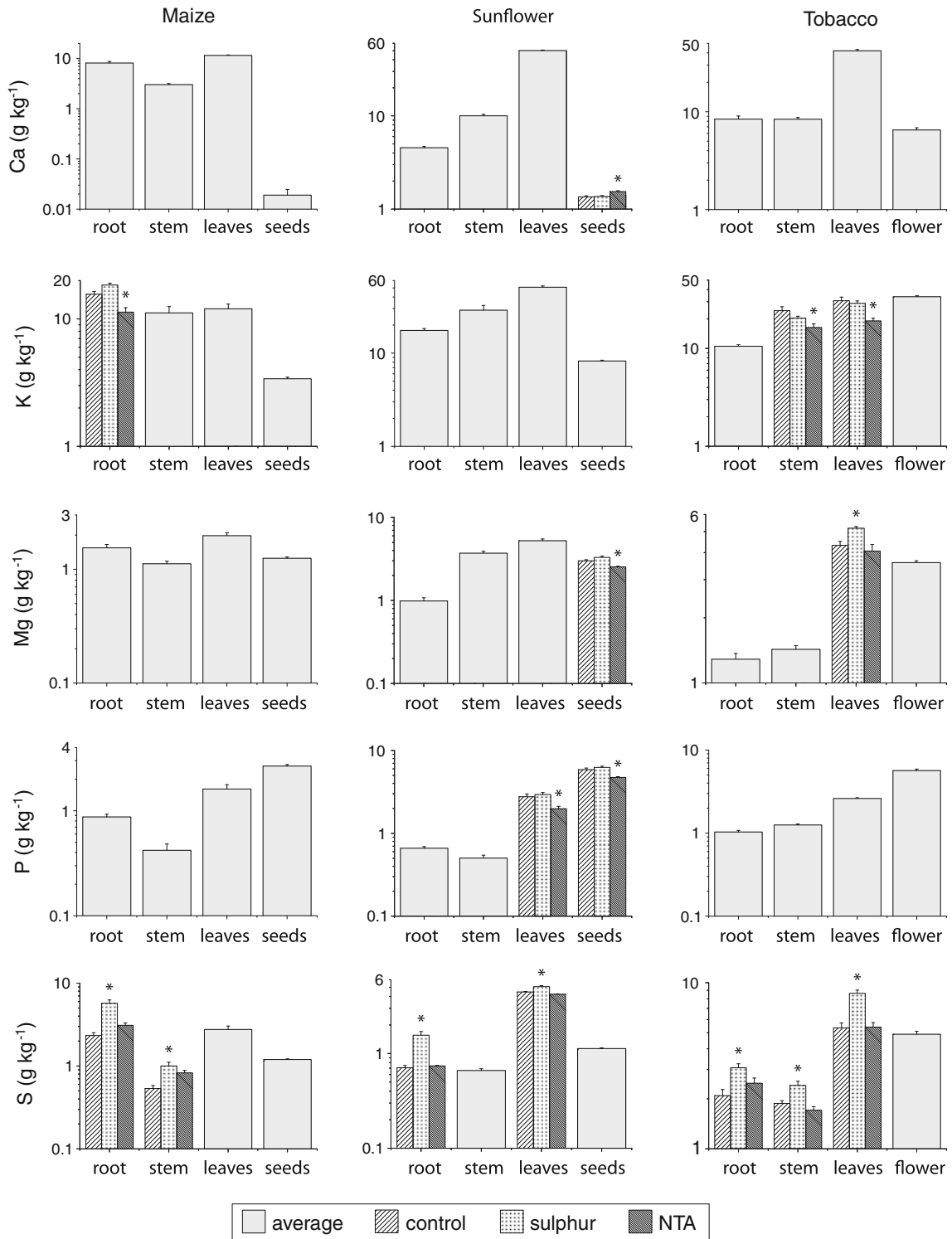
The allocation patterns of the micronutrients Cu, Fe, Mn, and Zn showed similar diversity as those of the macronutrients (Fig. 4). The allocation patterns of Cd were most similar to those of S, in particular for the vegetative parts, and resembled those of Zn and Mn (Fig. 5). In maize, the micronutrient concentrations were always highest in the roots and lower in stems and seeds than in the leaves. The Cd and Cu concentrations of maize seeds were below their

respective detection limits. Maize is known as a shoot excluder of Cd, meaning that the root-shoot translocation of Cd is strongly restricted (Marschner 1995). In contrast, sunflower and tobacco translocated Cd, Zn, Mn, Cu and Fe efficiently into their upper parts. In these two plant species, Cd and Zn preferentially accumulated in the leaves. In sunflower this was also the case for Mn.

Given that Cd preferentially accumulates in leaves compared to other aboveground plant parts, growing leafy vegetables may be riskier than producing grains for food or feed on Cd-contaminated soil (Alloway and Jackson 1991). However, for many crops it is the seeds that are consumed. A comparison of the element concentrations of maize and sunflower seeds with reference values given in the literature for human and animal nutrition is given in Table 3. Sunflower seeds contained normal concentrations of Ca, Fe and Zn. Copper, K, and Mg were slightly above the normal mean values. Maize had Ca and Cu concentrations far below the respective mean concentrations, whereas Mg and K exceeded reference values. The high K concentration of the maize grains is further evidence that the maize plants were not deficient in K, even though the average K concentration of the whole plant was below the respective reference value (as discussed above). There are no Swiss threshold values for concentrations of these elements in plant raw materials used for food or feed. Underwood and Suttle (1999) reported that the tolerable limit of Mg concentrations for ruminants was  $5 \text{ g kg}^{-1}$  dry matter by the US National Academy of Sciences.

There are no reported threshold values for K, however, a concentration of  $60 \text{ g K per kg}^{-1}$  fodder led to a decline of appetite and growth in calves (Underwood and Suttle 1999). Our measured Mg and K concentrations in maize grains of  $1.18 \text{ g kg}^{-1}$  and  $3.18 \text{ g kg}^{-1}$ , should not negatively affect animal health. Moreover, grain Fe, Mn, S were in the normal range, while grain Zn was slightly above.

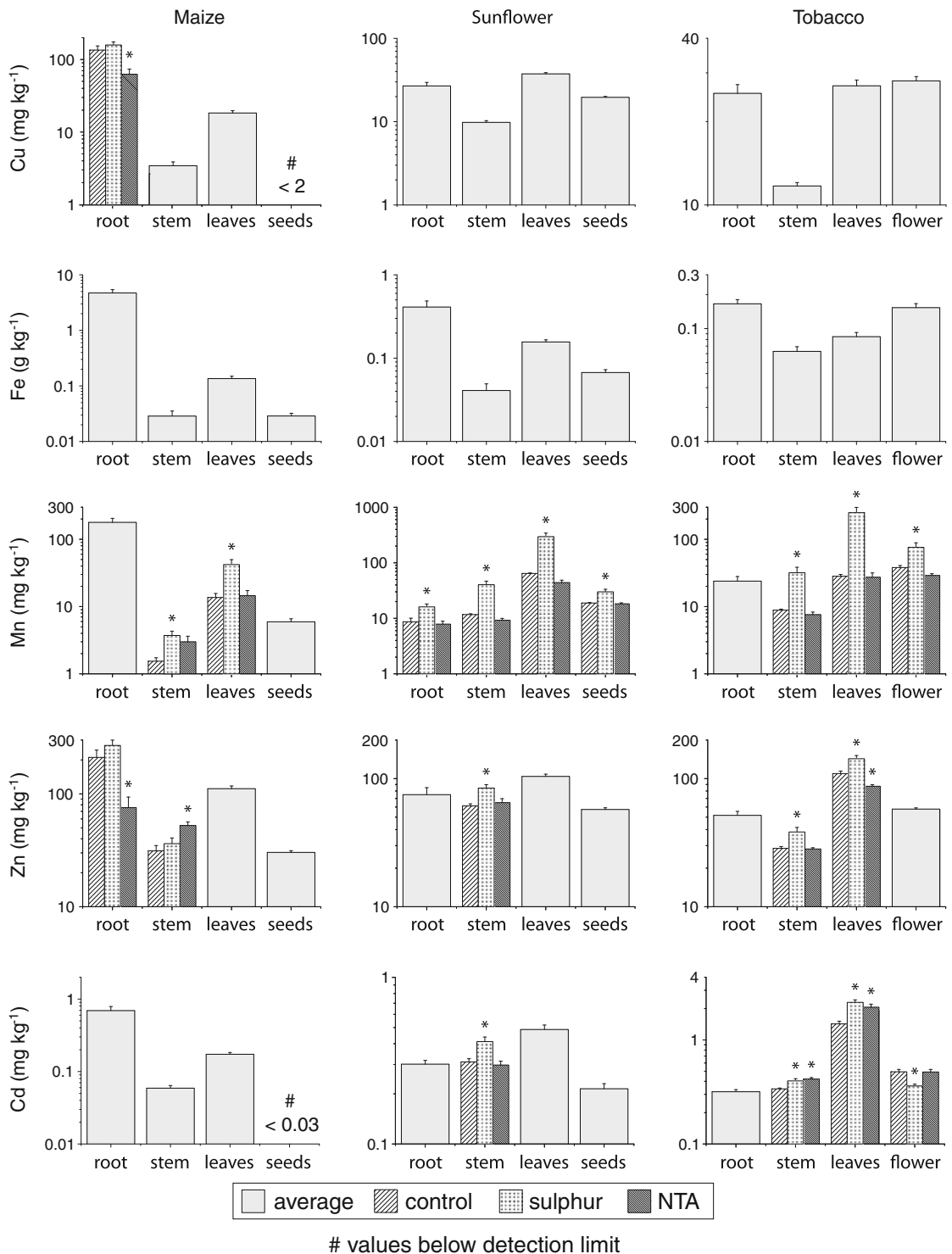
The mineral nutritional value of seeds depends on the bioavailability of the nutrient elements to humans and animals, rather than just on the total concentrations. In particular, excessive levels of P in seeds, when it is primarily present in form of phytate, may reduce the bioavailability of nutrients as well as contaminants. In our case, the P concentration was slightly above the normal range for seeds in both plants (Table 3). The concentration of Cd, the main



**Fig. 3** Allocation of the macronutrients Ca, K, Mg, P, and S in maize (left), sunflower (middle), and tobacco (right). The wide grey bars show the mean nutrient concentrations for those parts

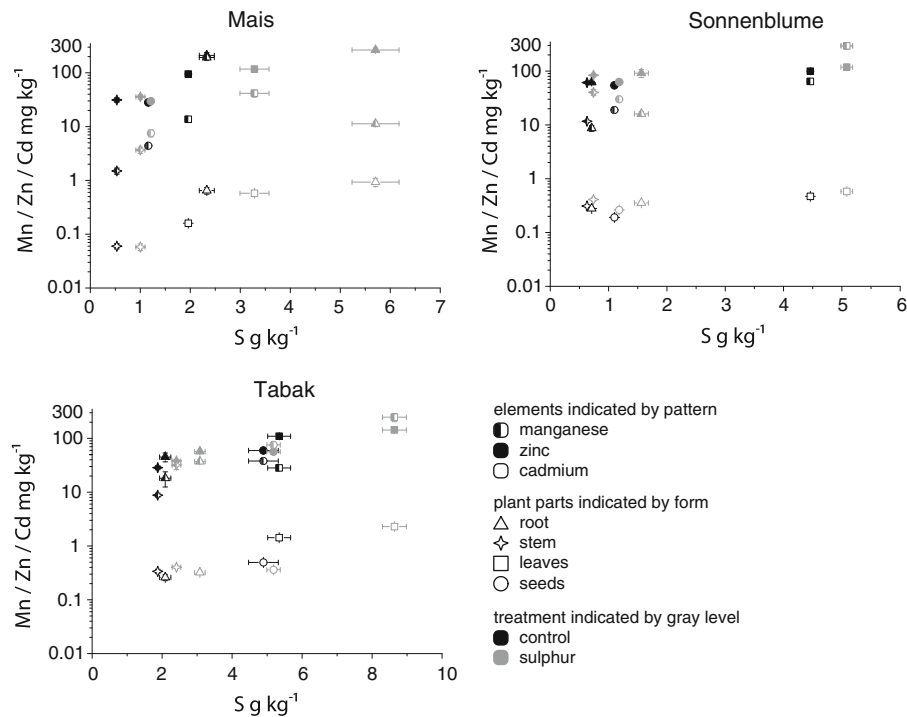
where no treatment effect was found. Asterisks indicate significant differences between treatment, and respective control. Error bars represent the standard errors of the means





**Fig. 4** Allocation of the micronutrients Cu, Fe, Mn, and Zn, and of Cd in maize (left), sunflower (middle), and tobacco (right). The wide grey bars show the mean concentrations for those parts

where no treatment effect was found. Asterisks indicate significant differences between treatment, and respective control. Error bars represent the standard errors of the means



**Fig. 5** Relations between S and Mn, Zn and Cd concentrations in plant material, with and without S treatment

**Table 3** Elemental concentrations of maize and sunflower seeds grown on the untreated contaminated soil and reference values for human or animal nutrition given in the literature

| Element | Unit                | Maize        |                                  | Sunflower    |                                  |
|---------|---------------------|--------------|----------------------------------|--------------|----------------------------------|
|         |                     | Measured     | Reference                        | Measured     | Reference                        |
| Ca      | g kg <sup>-1</sup>  | 0.02 (0.00)  | 0.5 <sup>b</sup>                 | 1.35 (0.03)  | 1.6 <sup>b</sup>                 |
| Cd      | mg kg <sup>-1</sup> | <0.031       | 0.1 <sup>c</sup> /1 <sup>d</sup> | 0.19 (0.01)  | 1.6 <sup>c</sup> /1 <sup>d</sup> |
| Cu      | mg kg <sup>-1</sup> | <2           | 9 <sup>b</sup>                   | 18.93 (0.31) | 14 <sup>b</sup>                  |
| Fe      | mg kg <sup>-1</sup> | 26.76 (5.47) | 25 <sup>b</sup>                  | 62.09 (0.61) | 68 <sup>b</sup>                  |
| K       | g kg <sup>-1</sup>  | 3.18 (0.09)  | 0.8 <sup>a</sup>                 | 8.64 (0.26)  | 6.4 <sup>a</sup>                 |
| Mg      | g kg <sup>-1</sup>  | 1.18 (0.03)  | 0.2 <sup>a</sup>                 | 3.00 (0.07)  | 3.87 <sup>a</sup>                |
| Mn      | mg kg <sup>-1</sup> | 4.39 (0.39)  | 5–8 <sup>e</sup>                 | 18.86 (0.29) |                                  |
| P       | g kg <sup>-1</sup>  | 2.47 (0.09)  | 3.4 <sup>b</sup>                 | 5.87 (0.22)  | 4.5 <sup>a</sup>                 |
| S       | g kg <sup>-1</sup>  | 1.16 (0.03)  | 1 <sup>e</sup>                   | 1.10 (0.02)  |                                  |
| Zn      | mg kg <sup>-1</sup> | 28.07 (0.87) | 21 <sup>b</sup>                  | 54.32 (1.05) | 48 <sup>b</sup>                  |

Standard errors of the means are in parentheses

<sup>a</sup> Tables of nutritional values of foodstuffs [values of sunflower seeds and polenta (Maisgriess)] (SwissFIR 2008)

<sup>b</sup> Tables of nutritional values of swine forage (values of sunflower seeds and maize corn) (ALP 2004)

<sup>c</sup> Threshold value for human nutrition [sunflower: value of oilseed, maize: value of cereals (übrige Getreide)] (FIV 1995)

<sup>d</sup> Threshold value for undesired substances in plant raw materials for animal feeding (FMBV 1999)

<sup>e</sup> Typical content in cereal grains (Underwood and Suttle 1999)

problem element in our soil with respect to food chain transfer, was below the threshold values in both, sunflower and maize seeds. Thus, they could be safely used at least as feed for animals.

#### Treatment effects on plant growth, element uptake and allocation

Neither the S nor the NTA applications significantly affected the biomass production of the plants (data not shown). They also had little effect on the uptake and allocation of the investigated macro- and micro-elements (Figs. 3, 4). Where it had an effect, the S treatment generally resulted in a slightly increased concentration of the affected element. Not surprisingly, the S treatment increased S concentrations in the plants. The greatest increase occurred in the roots of all three crops, but the S concentrations increased also in maize and tobacco stems as well as in sunflower and tobacco leaves, while no significant effects were found in the seeds and flowers.

Apart from S itself, Mn, Zn and Cd responded most strongly to the S treatment (Fig. 5). These elements showed similar allocation patterns and similar relationships to the S concentration in the various plant parts. Most conspicuous was the increase in Mn accumulation, which was observed in all three plant species, primarily in the stems and leaves, and in sunflower roots and seeds as well as in the flowers of tobacco (Fig. 4). Sulphur applications were also found by other authors to increase plant Mn concentrations (Juste and Solda 1988; Cimrin et al. 2008). In sunflower and tobacco, Zn was slightly increased in the stems, but not in the seeds. An S effect on leaf Zn was only observed in tobacco. The S treatment effects on Cd concentrations were similar to those on Zn in sunflower and tobacco, with the exception of the rather unexpected result that Cd was slightly decreased in tobacco flowers. Magnesium was increased in tobacco leaves (Fig. 3).

For a comparison with the results obtained in the previous study on the same experimental field (Fässler et al. 2009) we calculated the mean element concentrations for the whole above ground biomass from the values of the individual plant parts of the above ground biomass. In the previous study, the S treatment had led to increased Mg and Cd concentrations in tobacco and an increased Zn concentration in all three plants, which is consistent with what we

obtained in the present study. The doubling of the S application in 2006 had little additional effect on plant metal uptake.

The results of the S treatment are also in good agreement with those of Kayser (2000), who investigated the response of maize and tobacco upon the addition of elemental S in two different soils (a carbonate rich silty clay loam with pH 7.4 and a low carbonate sandy loam with pH 6.8). While S applications decreased the pH of both soils, plant accumulation of S and Mn was only increased in the soil with low CaCO<sub>3</sub>, indicating that not the pH effect but the increased availability of S was the dominating factor for the increase in Mn uptake. In agreement with our findings here, Kayser (2000) reported that the responses of plant Cd and Zn concentrations were similar to those of plant S and Mn concentrations.

The observed similarities in the accumulation and allocation patterns of Cd, Zn and Mn suggest that transport mechanisms common to all three of these elements played a role. Cadmium is taken up into plant cells among others through transporters belonging to the ZIP and Nramp families, which also transport Zn and Mn (Guerinot 2000; Pittman 2005).

The similarity in Zn and Cd allocation has been reported in other species. In *Arabidopsis halleri* it was not only found at the level of whole plant organs, but also of single cells (Küpper et al. 2000). Ma et al. (2005) also found that Cd and Zn had similar subcellular localisation in the leaves of *Thlaspi caerulescens*. The similarity of the two metals also results in competition for binding sites. This is one reason why not only positive correlations are found between Cd and Zn uptake by plants, but also antagonistic effects (Cosio et al. 2004). However, plants discriminate between Cd and Zn by producing ligands that are particularly specific for one of them. Studying Cd-stressed *Thlaspi caerulescens* plants Küpper et al. (2004) found that histidine was the main ligand for Zn, while SH-bearing ligands, such as phytochelatins, predominantly bound Cd.

Phytochelatins have a particularly high binding affinity for Cd compared to other metal ions and are known to be involved in the detoxification of Cd in plants (Satofuka et al. 2001; Cobbett and Goldsbrough 2002). As phytochelatins are rich in S, the increased S accumulation observed in the crop plants on the S-treated plots may not only have been a response to higher S-availability in the soil but also to

increased Cd stress due to the decrease in soil pH. This is consistent with the observation of an increased shoot S concentration in Cd-stressed *Arabidopsis halleri* plants by Küpper et al. (2000).

Competition in plant uptake was not only reported to exist between Cd and Zn (Cosio et al. 2004) but also between Cd and Mn. Hernandez et al. (1998) treated hydroponically grown *Pisum sativum* and *Zea mais* with 50  $\mu\text{M}$  Cd and found that Mn uptake was almost completely inhibited, while Peng et al. (2008) found that the addition of 9.1–5,000  $\mu\text{M}$  Mn to Cd-treated (10–50  $\mu\text{M}$ ) *Phytolacca americana* plants significantly reduced Cd accumulation and alleviated Cd toxicity in a hydroponic experiment. The increase in plant Mn concentrations on the S-treated plots may thus have counteracted a stronger increase in Cd uptake in our study.

In the few cases, where we observed an NTA effect on the uptake or allocation of the investigated elements, it consisted of a slight decrease, with few exceptions. There were decreases in shoot concentrations of K and Zn in tobacco and P in sunflower (Figs. 3, 4), while Zn was increased in maize stems. The decrease in shoot K accumulation of tobacco led to a concentration below the sufficiency range given for this plant by Bergmann (Table 3). In contrast to the effects of S applications, NTA had no effects on Mn concentrations, but decreased the K, Cu and Zn concentrations in maize roots. The decrease in root Zn combined with the increase in stem Zn in maize may indicate an enhanced translocation of Zn from the roots to the shoot with NTA treatment. In sunflower seeds, NTA decreased Mg and P, and increased Ca. Also, in contrast to the observed S effects, NTA effects on Cd differed from those on Zn. The accumulation of Cd was increased in tobacco leaves and stems by NTA, but was unaffected in all other cases. While the increased Zn concentration in maize stems is consistent with the results obtained in previous years (Fässler et al. 2009), the increased Cd accumulation in tobacco was new and may be due to the doubling of the applied NTA concentration compared to the latter study.

#### Implications for agricultural crop production

Although the  $\text{HNO}_3$ -extractable soil Cd and Zn concentrations exceeded the respective OIS guide values and in the case of Cu, the trigger value, the

concentrations of these elements in edible parts of all the plants in all treatments were below the values considered problematic for consumption by humans or animals (Tables 2, 3). Plant metal concentrations exceeding regulatory values were only observed in the case of tobacco, where leaf Cd concentrations exceeded the Swiss threshold value of 1  $\text{mg kg}^{-1}$  for undesired substances in raw materials for animal feeding (FMBV 1999), in all treatments.

Although tobacco is not used as a food plant, the accumulation of Cd in tobacco may exacerbate the harmful effects of smoking, which is the main source of Cd intake by humans (Bernhard et al. 2005). Leaf concentrations of tobacco used for cigarette production are reported to vary between 0.5 and 5  $\text{mg kg}^{-1}$  on uncontaminated soil (Lugon-Moulin et al. 2004). The leaf Cd concentrations found in our experiment on the untreated soil were in the lower part of this range and were still in this range after S or NTA treatments. To be on the safe side, however, tobacco plants may be used for energy production instead (Meher et al. 1995). The unexpected finding that Cd accumulation in the tobacco flowers was slightly decreased in the S treatment indicates that testing the effect of S amendments on the seeds and fruits of related plants, such as tomato (*Solanum lycopersicum*) or chilli peppers (*Capsicum* sp.) may be an interesting avenue for future research.

The elevated total soil metal concentrations are still relevant for crop contamination risks arising from the deposition of suspended soil particles on plant surfaces due to wind erosion or rain splash. We compared our plant concentrations with data obtained from plants grown the same field but harvested in usual agricultural manner, i.e. they were passed through a chaff cutter directly on the field without being washed (data not shown). The Cu concentrations were significantly lower in all three washed compared to the unwashed crops (maize 52%, sunflower 12% and tobacco 22%). Zinc concentrations in maize and sunflower were also significantly lower in the washed plants (maize 16%, sunflower 22%). No washing effect was found for Zn in tobacco and for Cd in all three plants. If economically feasible, washing the plants before feeding them to animals would thus reduce the animals' exposure to Cu and Zn. As the amount of suspended soil particles on the plant surface certainly decreases with the distance to the soil surface, an increase in the cutting height might also reduce the contamination.

Our results show that moderate concentrations of contaminating metals do not necessarily render agricultural soils unsuitable for the production of food or fodder plants. However, when planting crops on contaminated soil, it is important to monitor and control the availability of the soil contaminants to potential crop plants. Heavy metal concentrations in plant tissues may increase over time in response to changes in acidity, organic matter and other soil properties, thereby taking particular account of the allocation of potentially toxic elements in the harvested plant parts. In the case of the soil investigated here, all three tested crops are realistic options to be used in a sustainable phytomanagement of the land, with or without S treatment.

## Conclusions

The studied soil contaminants at Witzwil were predominantly accumulated in the leaves and/or roots, rather than the seeds. Thus sunflower seeds or maize grain could be safely used as food or animal feed. In contrast, the production of leafy vegetables may result in these toxic elements presenting a health risk to humans or animals. Besides of the increase in Mn accumulation by all three tested crop plants after S application, both treatments (S and NTA) had only slight or no effects on the uptake and allocation of plant nutrients and Cd. Thus, there is little benefit of these treatments for phytoextraction purposes at this site, even though the application doses were doubled compared to the previous years. The use of sunflower, maize and tobacco without treatment, on the other hand, provides valuable biomass while the environmental risks posed by the soil contaminants are gradually reduced.

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