Antimony uptake and toxicity in sunflower and maize growing in Sb^{III} and Sb^{V} contaminated soil

Martin Tschan · Brett Robinson ·
C. Annette Johnson · Annina Bürgi ·
Rainer Schulin

Abstract Using pot experiments, we investigated the uptake of antimony (Sb) by sunflower (*Helianthus annuus* L. cv. Iregi), and maize (*Zea mays* L. cv. Magister) in two different soils, a potting mix and an agricultural soil. In one treatment Sb was added to the experimental soils as $KSb(OH)_6$ ("Sb^{V}-treatment") and in the other as Sb_2O_3 ("Sb^{III}-treatment"). Soluble soil Sb concentrations were linearly related to the applied Sb rates, ranging from 0.02 (controls) to 175 mg L^{-1} soil solution. Accumulation of Sb tended to be slightly higher in the Sb^{V} treatment in sunflower, while no difference in Sb uptake between the two Sb treatments was found in maize. The half maximal effective concentration (EC_{50}) values derived from the dose-response curves were higher for the Sb^{V} than for the Sb^{III} treatment when they were related to soluble soil Sb concentrations, but differences became insignificant when they were related to shoot Sb concentrations. Maize was substantially more sensitive to Sb toxicity than sunflower, indicating physiological differences in Sb tolerance between the two plant species. Our results show that on soils with high Sb contamination, as often found in shooting ranges, plants may suffer from Sb toxicity.

Introduction

The concentration of antimony (Sb) in soils is generally below 10 mg kg^{-1}, with most soils having <1 mg kg^{-1} Sb (Johnson et al. 2005; Kabata-Pendias and Pendias 1984). Higher concentrations of Sb in soil are usually a result of human activities. Due to its many industrial uses, e.g. in fire retardants, semiconductors, and as an agent for metal hardening, Sb is released into the environment in increasing amounts (Filella et al. 2002). High soil Sb concentrations are often found in areas affected by Sb-mining activities (210 to 360 mg kg^{-1} in Ainsworth et al. 1990; 192 to 15,112 mg kg^{-1} in Baroni et al. 2000; 31 to 5,986 mg kg^{-1} in Pratas et al. 2005), along roadsides (1.2 to 8.7 mg kg^{-1} in Amereih et al. 2005) and particularly in shooting ranges (Johnson et al. 2005). The latter is due to the fact that

M. Tschan (✉) · A. Bürgi · R. Schulin
Eidgenössische Technische Hochschule (ETH) Zurich,
Institute of Terrestrial Ecosystems ITES,
Universitaetstrasse 16,
CH-8092 Zurich, Switzerland
e-mail: martin.tschan@alumni.ethz.ch

B. Robinson
Agricultural and Life Sciences Division,
Lincoln University,
PO Box 84, Canterbury, New Zealand

C. A. Johnson
Swiss Federal Institute for Environmental
Science and Technology (EAWAG),
CH-8600 Dübendorf, Switzerland

Sb is used to harden lead bullets, making up 1–7% of their weight (Rooney et al. 1999). Soil contamination by Sb in shooting ranges is an important environmental problem in Switzerland, where more than 2,000 shooting ranges are scattered over the country (total area of only 41,000 km^2). Concentrations of up to 2,400 $mg kg^{-1}$ have been found in stop butt soils (Gresch and Wettstein 2002).

While lead (Pb) is the main contaminant in shooting range soils on a mass basis, soil contamination by Sb may be of greater concern, given that Sb is much more soluble than Pb in the near-neutral pH range characteristic of many Swiss soils (Blaser et al. 2008) and that the toxicity of Sb to humans and animals is considered comparable to its sister element arsenic (Belzile et al. 2001; Bowen 1979). Similar to As, inorganic forms of Sb are generally more toxic than organic Sb compounds (Gebel 1997). Likewise, Sb toxicity also depends on its oxidation state, with Sb^{III} being much more toxic than Sb^V (Gurnani et al. 1994). In mammal cells Sb^{III} compounds were found to be ten times more toxic than Sb^V compounds (Krachler et al. 2001).

In soils Sb is usually present as Sb^{III} and Sb^V. Antimony entering soil as elemental Sb, for example as part of the lead amalgam of bullets in the case of shooting range soils, is rapidly oxidized to Sb^{III} and Sb^V, depending on soil pH and redox conditions (Johnson et al. 2005). Trivalent antimony is present in soil solution as antimonite, i.e. as the neutral species $Sb(OH)_3$ between pH 2.5 and pH 10.8, the soil pH range relevant under normal environmental conditions (Baes and Mesmer 1986). Apart from the oxidation of elemental Sb entering soil in the form of metal alloys, Sb^{III} in soil can originate from flame retardants, which contain Sb_2O_3, or by reduction of Sb^V. Pentavalent antimony occurs in soil solution as antimonate, i.e. as the oxyanion $Sb(OH)_6^-$ between pH 2.7 and pH 14 (Baes and Mesmer 1986). Iron plays an important role in the redox chemistry of Sb. In alkaline conditions Fe^{II} can reduce Sb^V, while in acidic to neutral conditions the oxidation of Fe^{II} with oxygen can produce intermediate products that oxidise Sb^{III} to Sb^V (Leuz 2002; Leuz et al. 2006a). The oxidation of Sb^{III} is also catalyzed when sorbed to amorphous Fe- and Mn-oxyhydroxides, for which Sb^{III} has a strong affinity (Belzile et al. 2001; Blay 2000; Leuz et al. 2006b). Oorts et al. (2008) found that more than 70% of the Sb added in solution as dissolved antimony trioxide to a soil was oxidized to antimonate within 2 days.

The neutral molecule $Sb(OH)_3$ was found to be taken up by bacteria (Escherichia coli), yeast (Saccharomyces cerevisiae) and protozoa (Leishmania major and Leishmania tarentolae) through aquaglyceroporins. Thus, it is likely that aquaglyceroporins can also mediate the uptake of $Sb(OH)_3$ by plants. Conversely, although the mechanisms of Sb^V uptake by plants are unclear (Filella et al. 2007), it is unlikely that antimonate can pass through aquaporins, because of its negative charge. Furthermore, the observation by Tschan et al. (2008) that phosphate did not affect antimonate uptake by sunflower and maize in hydroponic experiments indicates that antimonate is not taken up by plants via phosphate transporters like arsenate (Asher and Reay 1979; Gulz et al. 2005; Woolson et al. 1973). Tschan et al. (2009a) found that antimonate is accumulated by plants approximately in proportion to soil soluble Sb over a concentrations range of several orders of magnitude, suggesting that antimonate uptake is dominated by a non-selective pathway, possibly an apoplastic bypass of the endodermis barrier separating the outer parts of a plant root from the central root cylinder with the vascular bundles. This latter pathway would also be available for antimonite.

Given the likelihood that Sb is taken up by plants from soil solution both as antimonate and also as antimonite, the influence of oxidation state on uptake rate and phytotoxicity needs further examination. Considering that even under oxidizing conditions substantial fractions of soil Sb may be present as Sb^{III} (Belzile et al. 2001), this question is particularly relevant for Sb-contaminated shooting range soils, where bullet weathering and oxidation of Sb(0) first lead to Sb^{III} before it is further converted to Sb^V. Thus, the objective of this study was to investigate how the form in which Sb was added to soil would affect its uptake by plants and its phytotoxicity. For this purpose we performed pot experiments in which two crop plant species, maize and sunflower, were grown on two different soil substrates, an agricultural topsoil and a commercial potting mix, to which Sb was added either as antimonite or as antimonate at various concentrations.

Material and methods

Sunflower (Helianthus annuus L. cv. Iregi) and maize (Zea mays L. cv. Magister) in 250-mL pots were grown in a climate chamber (photoperiod 16 h, day/night

temperature 22/14°C, light intensity 11,000 lux). Two soils were used: a standard potting mix (obtained from Migros Co-operative, Zürich), consisting of garden soil enriched with compost (organic carbon content: 229 ± 27 g kg^{-1}, pH in CaCl$_2$ extract: 7.0 ± 0.1) and soil from the plough layer of an agricultural field at Birr (Canton Aargau) on the Swiss plateau (organic carbon content: 15 ± 1.4 g kg^{-1}, pH in CaCl$_2$ extract: 6.6 ± 0.1). Either antimonite ("Sb^{III} treatment") or antimonate ("Sb^V treatment") was added by mixing granular Sb$_2$O$_3$ or KSb(OH)$_6$, respectively, with the sieved and dried soil substrates. Apart from the control (no Sb added), the applied total Sb concentration levels were 156, 313, 625, 1,250, 2,500, 5,000, and 10,000 mg kg^{-1} for the potting mix and 20, 39, 78, 156, 313, 625, 1,250, 2,500, and 5,000 mg kg^{-1} for the agricultural soil. (Note: In order to avoid confusion, we will never use the term "Sb treatment" to denote the application of specific Sb concentration levels in the following. This term will only be used to refer to the application of Sb in the two different chemical forms of antimonite and antimonate.) In a preliminary experiment we tested for eventual phytotoxic effects of the high amounts of potassium (K) added with the Sb applications to the soil. However, even at a rate of 3,780 mg kg^{-1}K applied either as K$_2$SO$_4$ or KH$_2$PO$_4$ to the soil, we found no negative effect on plant growth.

Regularly watered, the mixtures were left to equilibrate for 2 weeks and then, after taking samples for chemical analysis (one composite sample per batch), filled into the pots. Three replicates were prepared for each concentration. Three seeds of either maize or sunflower were planted in each pot, of which only one was left to grow after a week. Plants were harvested after 4 weeks of growth, separated into roots and shoots, carefully washed with de-ionized water, oven-dried for 48 h at 65°C, weighed and then digested for chemical analysis using aqua regia (Sample weight for digestion was 0.2 g which was digested in 2 ml HNO$_3$, 6 ml HCl, 2 ml H$_2$O$_2$ and 8 ml H$_2$O) in closed Teflon vessels (2 h at room temperature and then for 30 min in a microwave oven (EM-2, Lavis ETHOS, MLS Microwave Laboratory Systems GmbH, Leutkirch i. A., Germany) at 100°C).

Soil samples were oven-dried at 65°C for 1 week, weighed and then stored at 4°C until they were analysed. Soluble soil Sb concentrations relating to soil solution were determined by extraction with potassium nitrate as described by Tschan et al. (2009a): Subsam-ples of 5 g were mixed with 12.5-mL aliquots of a 0.1 mol L^{-1} potassium nitrate solution in polypropyl-ene bottles. The bottles were tightly closed and longitudinally shaken for 2 h with a frequency of 120 min^{-1} and an amplitude of 55 mm (KS 250, Janke & Kunkel IKA Labortechnik GmbH, Staufen i. Br., Germany). The resulting slurries were left for 10 min to settle, before the supernatants were collected using 60-mL single-use syringes and filtered through 45-µm membrane filters. The filtrates were collected in 20-mL volumetric flasks containing 0.8 mL of 65% nitric acid.

The plant and soil extracts from the potting mix experiments were analysed for Sb by means of hydride generation atomic fluorescence spectroscopy (HG-AFS) (10.055 Excalibur Millenium System, PSAnalytical, Orpington, Kent, UK) and those from the experiments with agricultural soil by means of stripping voltametry with a mercury anode (797 VA Computrace, Metrohm, Herisau, Switzerland). Total soil Sb concentrations were measured by means of XRF (X-Ray fluorescence spectrometry) (Spectro). As reference material for the analysis of plant samples, we used Virginia tobacco leaves (CTA-VTL-2) obtained from LGC Standards (Wesel, Germany). The mean ± standard error of our measurements was 0.295 ± 0.006 mg kg^{-1} for HG-AFS and 0.316 ± 0.030 for stripping voltametry, which agreed well with the certified values (0.312 ± 0.025 mg kg^{-1}) of the standards. Statistical analyses (ANOVA and regres-sion) were performed using SPSS 17.0 (SPSS 2008). Slopes of regression lines were compared by means of t-tests (Sachs 2004).

Results

Soluble soil antimony concentrations

The soluble Sb concentrations determined after the initial equilibration phase prior to planting were significantly correlated to the total Sb concentrations for both treatments and soil substrates, showing linear relationships on log-log scales (Figs. 1 and 2). The form in which Sb had been added showed no effect on this relationship in the agricultural soil, but there was a clear treatment effect in the case of the potting mix. Sb was more soluble in the Sb^V than in the Sb^{III} treatment with potting mix, suggesting that a substan-tial fraction of the added Sb^{III} was still present in that form, bound to the soil matrix. Interestingly, the
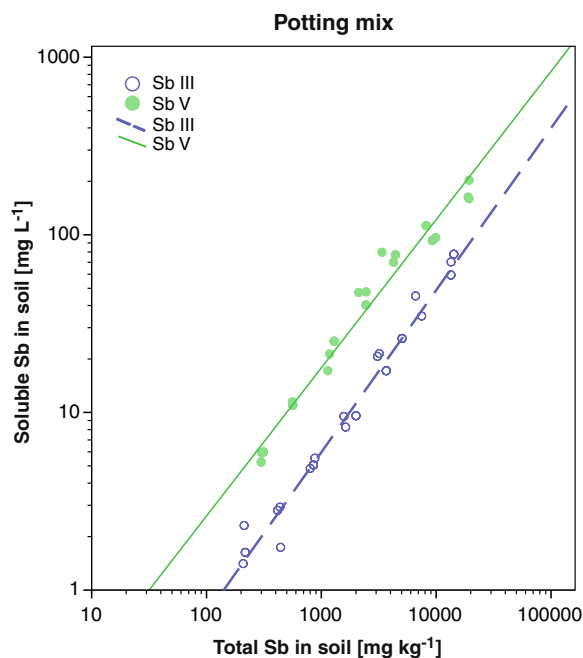
Fig. 1 Concentrations of soluble (mg L⁻¹ soil solution) and total Sb (mg kg⁻¹ dry soil) in the potting mix after adding various amounts of either antimonite (Sb^{III} treatment) or antimonate (Sb^{V} treatment). *Points* represent experimental data; *lines* were determined by linear regression on the log-transformed data

relationships between total and soluble soil Sb was very similar for the two substrates in the Sb^{III} treatment, meaning that Sb solubility was about the same in the two substrates where Sb had been added as Sb^{III}, whereas there was a pronounced difference in Sb solubility between the two substrates where Sb had been added as Sb^{V}. Given that there was no solubility difference between the two Sb treatments in the agricultural soil, the main difference is the elevated Sb solubility in the potting mix in the Sb^{V} treatment as compared to the other treatments.

The slopes of the log-log relationships did not differ significantly from 1 for both soils and both treatments, which means that soluble Sb concentrations were approximately proportional to total soil Sb concentrations in all cases. Thus, the partitioning of Sb between soil matrix and soil solution could be expressed by a constant distribution coefficient K_D (K_D = concentration sorbed / concentration dissolved). The K_D values found here were 195.0±13.2 Lkg⁻¹ for the Sb^{III} treatment and 169.8±9.9 Lkg⁻¹ for the Sb^{V} treatment in the case of the agricultural soil, and 144.5±12.5 Lkg⁻¹ for the Sb^{III} treatment and 33.9±6.2 Lkg⁻¹ for the Sb^{V} treatment in the case of the potting mix.

Phytotoxicity and uptake of antimony

At the highest Sb concentrations applied in this study, toxicity symptoms such as reduced growth, chlorosis and leaf necrosis were observed in the two plants. Maize also showed anthocyane coloring of the stem base. Based on the shoot biomass data shown in Figs. 3 and 4, maize appeared to be more sensitive to Sb in soil than sunflower. To quantify Sb toxicity on growth, we fitted the following 2-parameter empirical model commonly used in toxicology to the recorded dose-response curves:

$$DW = exp(b + a*c) \tag{1}$$

where *DW* is the dry weight of the aboveground biomass, c denotes the soluble Sb concentration of the soil, and a and b are fitting parameters. For comparison, because of its higher flexibility, we also fitted the following 3-parameter logistic model to the data:

$$DW = (1/u + v(w^c))^{-1} \tag{2}$$

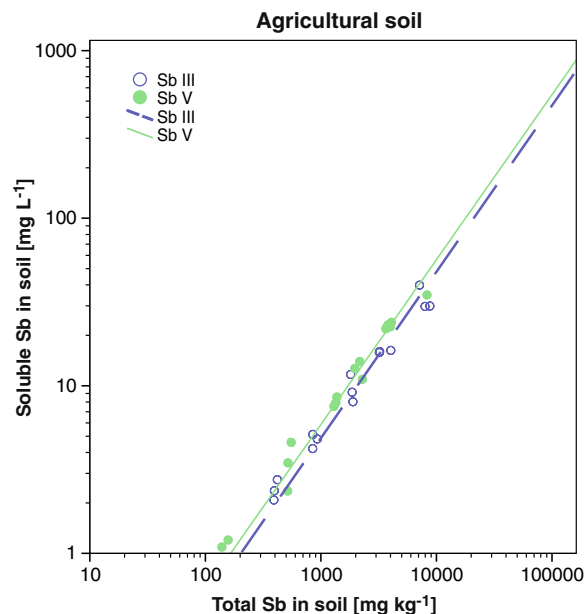where *u*, *v* and *w* are the fitting parameters. The parameter *u* corresponds to exp(*b*) in the 2-parameter

Fig. 2 Concentrations of soluble (mg L⁻¹ soil solution) and total Sb (mg kg⁻¹ dry soil) in the agricultural soil after adding various amounts of either antimonite (Sb^{III} treatment) or antimonate (Sb^{V} treatment). *Points* represent experimental data; *lines* were determined by linear regression on the log-transformed data
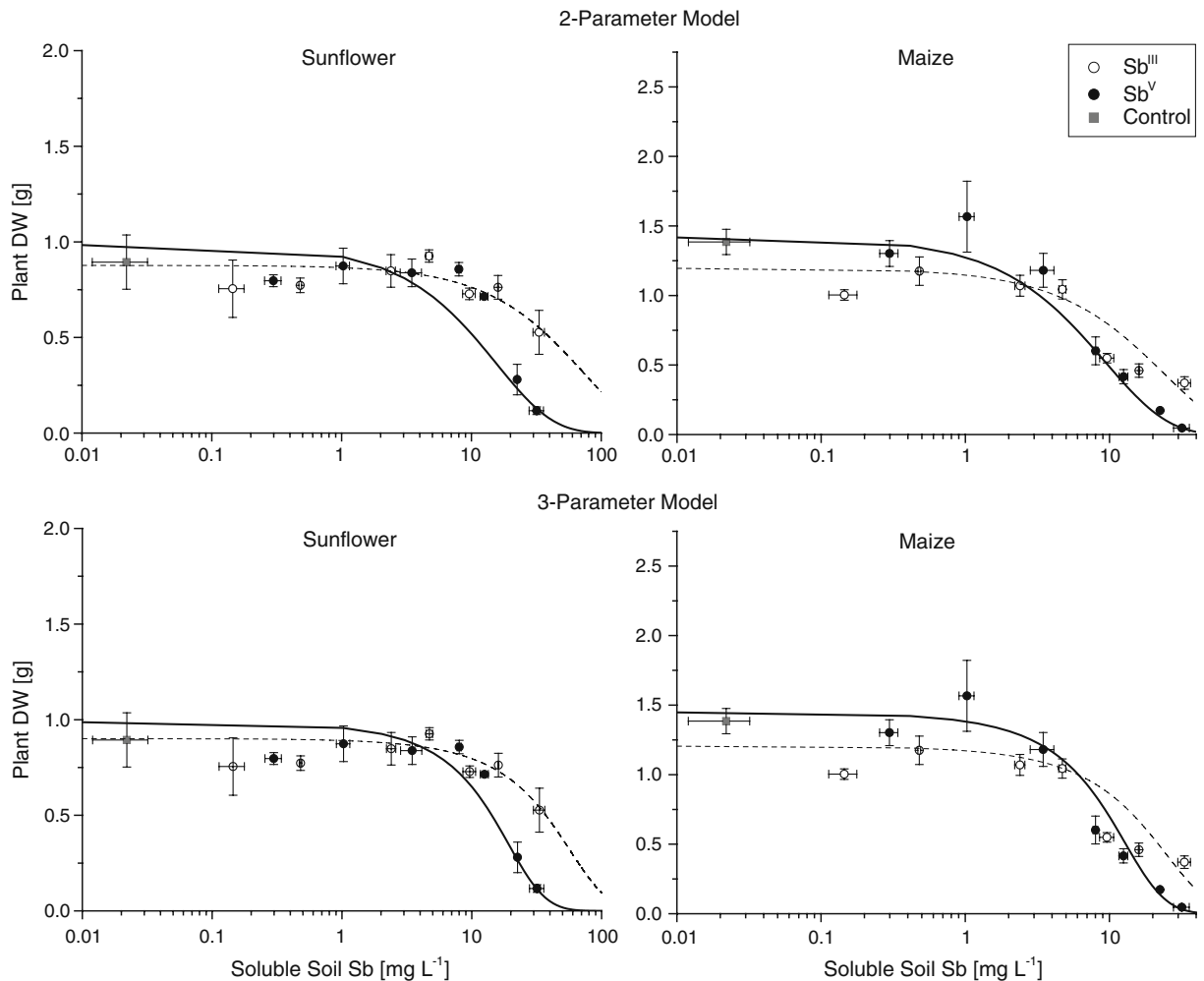
Fig. 3 Shoot biomass (g per plant) of 4-weeks old sunflower and maize plants as a function of the concentration of soluble Sb (mg L^{-1} soil solution) in agricultural soil to which various amounts of either antimonite (Sb^{III} treatment) or antimonate (Sb^{V} treatment) had been added. *Points* represent averages of 3–4 replicates each. *Error bars* represent standard errors. *Lines* are model curves fitted to the experimental data using the 2-parameter model given by Eq. 1 and the 3-parameter model given by Eq. 2 in the text

model: both represent the dry mass DW_o produced in absence of Sb, i.e. at c=0. The fitted models were used to calculate EC$_{50}$ values, i.e. the values of the soluble soil Sb concentrations at which growth was reduced to 50% of DW_o. Both models gave very similar results (Tables 1 and 2). The results, which are listed in Table 1, indicate that both plants were more sensitive to soluble soil Sb in the Sb^{V} than in the Sb^{III} treatment, although the difference was only significant for sunflower on the agricultural soil. Furthermore they reveal that soluble soil Sb was approximately 5 times more effective in reducing growth in the agricultural soil than in the potting mix. In the potting mix

experiment, no toxicity was observed in sunflower even at the highest rate of Sb^{III} application. Table 2 shows that the Sb treatment effect on EC$_{50}$ became insignificant, when DW was analysed as a function of shoot Sb concentrations, indicating that there were little or no substantial differences between the two Sb treatments in the oxidation state of the Sb that had been taken up by the plants and translocated into the shoots. In the case of maize, the disappearance of a significant difference between the two Sb treatments was primarily due to a larger scatter in the latter dose-response curves compared to the curves relating to soluble soil Sb. In contrast to the reduction in the difference
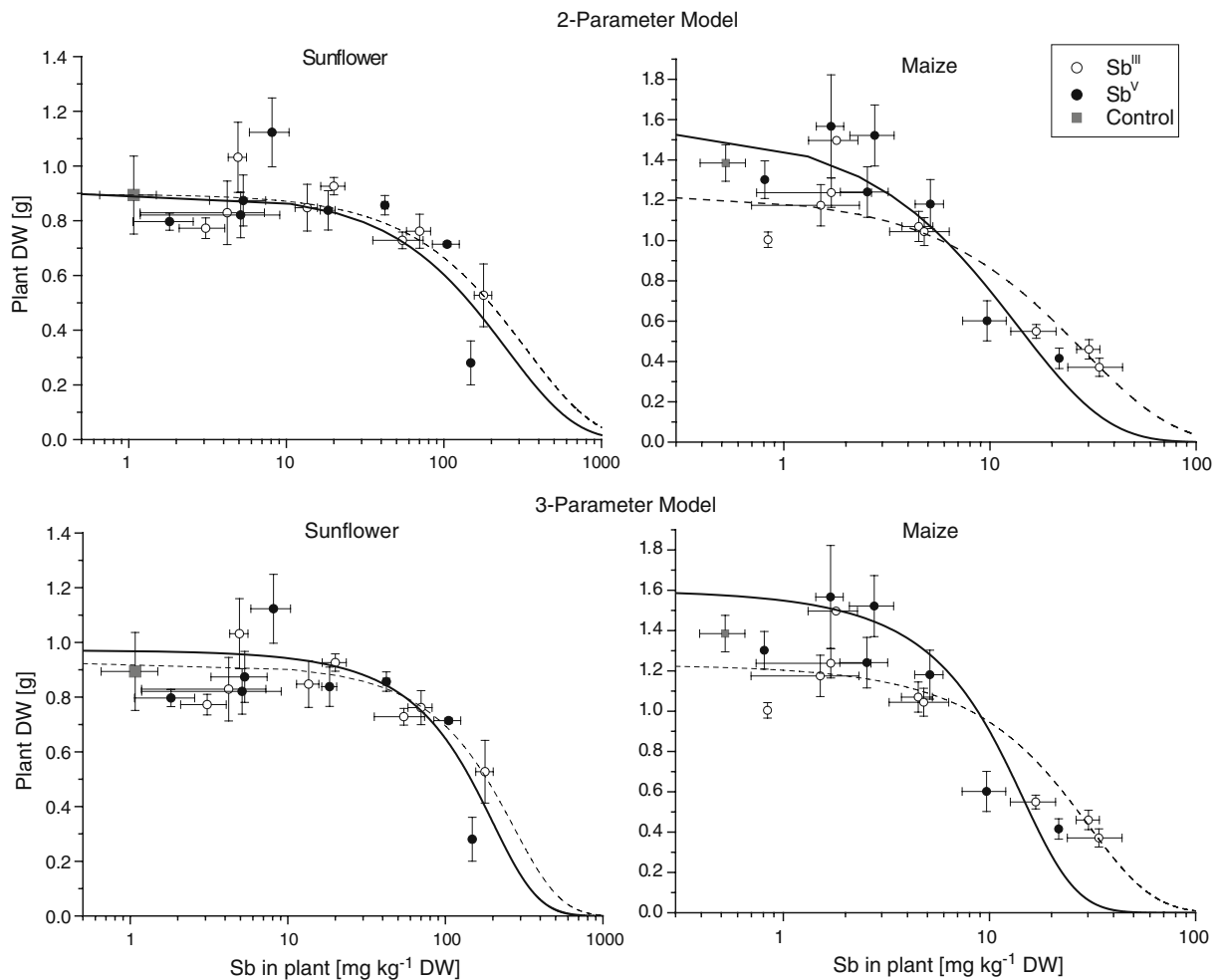
Fig. 4 Shoot biomass (g per plant) of 4-weeks old sunflower and maize plants as a function of the concentration of plant Sb (mg kg^{-1} dry weight) in agricultural soil to which various amounts of either antimonite (Sb^{III} treatment) or antimonate (Sb^{V} treatment) had been added. *Points* represent averages of 3–4 replicates each. *Error bars* represent standard errors. *Lines* are model curves fitted to the experimental data using the 2-parameter model given by Eq. 1 and the 3-parameter model given by Eq. 2 in the text

between Sb treatments effect, the differences in growth response to Sb between the two plant species became more pronounced when their shoot biomass was related to the concentration of Sb in the shoots (Fig. 4). This finding shows that maize is physiologically more sensitive to Sb than sunflower, and that the difference between the two plant species is not due to different accumulation rates.

Figures 5, 6 and 7 show that Sb accumulation in the shoots was linearly related to the soluble soil Sb concentration on a log-log scale. The parameter values given in Table 3, which were determined by linear regression on the logarithms of the respective concentration values, excluding plants that showed

clear toxicity symptoms, reveal that the slopes of the lines were close to 1 for sunflower on the agricultural soil in both Sb treatments (Table 3). This means that Sb accumulation by sunflower was approximately proportional to the soluble soil Sb concentration. The relationship was similar in both Sb treatments, with a tendency for higher Sb accumulation by sunflower in the Sb^{V} treatment. Also, we found no significant difference in Sb uptake between the two Sb treatments in maize, although here a treatment effect may have been masked by the larger scatter in the data compared to sunflower. The slopes of the log-log regression line between shoot and soluble soil Sb concentrations, however, were smaller for maize than

Table 1 EC_{50} values for sunflower and maize growth reduction due to toxicity of soil Sb applied to potting mix or agricultural soil either as antimonite (Sb^{III}) or antimonate (Sb^{V}). The EC_{50} here is the concentration of soluble soil Sb (mg L^{-1}) at which growth is reduced to half of the growth in absence of Sb. The EC_{50} values and standard errors (SE) were derived from the best-fit model dose-response curves to the experimental data using the two models defined by Eq. 1 (2-parameter model) and 2 (3-parameter model), as described in the text

Substrate	Model	Treatment	H. annuus		Z. mays	
			EC_{50}	SE	EC_{50}	SE
Potting mix	2-parameter model	Sb III	[a]	[a]	85.8	57.8
		Sb V	52.6	10.4	35.9	3.2
	3-parameter model	Sb III	[a]	[a]	83.0	63.5
		Sb V	62.0	24.7	46.8	17.1
Agricultural soil	2-parameter model	Sb III	48.3	14.2	16.6	1.5
		Sb V	10.8	0.9	6.5	0.2
	3-parameter model	Sb III	42.7	17.9	18.1	2.7
		Sb V	14.4	3.8	9.7	2.2

[a] no toxicity effect even at the highest treatment level

for sunflower. For both Sb treatments they were clearly less than 1 for maize on the agricultural soil, which means that the accumulation efficiency decreased with increasing concentration of Sb. As a result maize also accumulated less Sb than sunflower at high soil Sb concentrations, while there was no difference at low soil Sb concentrations.

Similar linear relationships between plant and soluble soil Sb as for the agricultural soil were also obtained for the potting mix. Although Sb was more soluble in the Sb^{V} than in the Sb^{III} treatment (Fig. 1), this difference did not clearly translate into Sb uptake by the plants. Uptake of Sb by sunflower was less at

Table 2 EC_{50} values for sunflower and maize growth reduction due to toxicity of soil Sb applied to agricultural soil either as antimonite (Sb^{III}) or antimonate (Sb^{V}). The EC_{50} here is the concentration of Sb (mg kg^{-1} DW) in plant shoots at which growth is reduced to half of the growth in absence of Sb. The EC_{50} values and standard errors (SE) were derived from the best-fit model dose-response curves to the experimental data using the two models defined by Eq. 1 (2-parameter model) and 2 (3-parameter model), as described in the text

Model	Treatment	H. annuus		Z. mays	
		EC_{50}	SE	EC_{50}	SE
2-parameter model	Sb III	231.3	61.1	19.9	2.2
	Sb V	193.2	64.1	9.5	1.5
3-parameter model	Sb III	205.0	77.0	21.9	4.1
	Sb V	150.7	56.2	11.3	7.8

low soil Sb concentrations from the potting mix than from the agricultural soil. However, as Sb accumulation increased slightly more than proportional with soluble soil Sb concentration, this difference disappeared at high soil Sb concentrations in potting mix while Sb accumulation was proportional to soluble soil Sb on agricultural soil. Figure 7 shows that uptake in leaves and stems was similar to the uptake in the whole shoot. While there was no significant difference between Sb^{III} and Sb^{V} treatments, Sb accumulation was two- to threefold higher in leaves than in stems. Unfortunately, the data for the maize plants grown in the potting mix were much more scattered than for the maize plants grown in the agricultural soil.

Discussion

As mentioned in the introduction a number of authors have reported rapid oxidation of Sb^{III} to Sb^{V} in soil or soil mineral suspension, in particular Fe and Mn oxyhydroxides (Belzile et al. 2001; Oorts et al. 2008). Belzile et al. (2001) found that the oxidation of dissolved Sb^{III} to Sb^{V} followed a pseudo-first order rate kinetics and was complete within a few days in presence of suspended amorphous Fe and Mn oxyhydroxides at neutral to weakly alkaline pH, while the rate was reduced at lower pH, which they attributed to a decreased stability of the oxyhydroxides. Oxidation
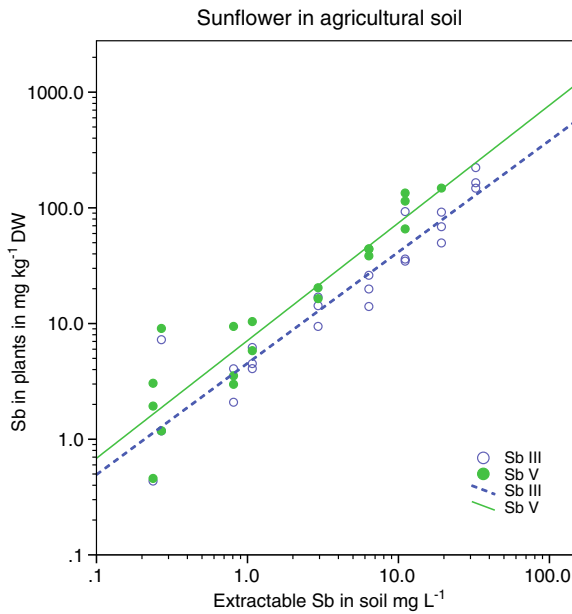
Fig. 5 Accumulation of Sb in 4-weeks old sunflower plants as a function of the concentration of soluble Sb (mg L^{-1} soil solution) in agricultural soil to which various amounts of either antimonite (Sb^{III} treatment) or antimonate (Sb^{V} treatment) had been added. *Points* represent experimental data; *lines* were determined by linear regression on the log-transformed data

Fig. 6 Accumulation of Sb in 4-weeks old maize plants as a function of the concentration of soluble Sb (mg L^{-1} soil solution) in agricultural soil to which various amounts of either antimonite (Sb^{III} treatment) or antimonate (Sb^{V} treatment) had been added. *Points* represent experimental data; *lines* were determined by linear regression on the log-transformed data
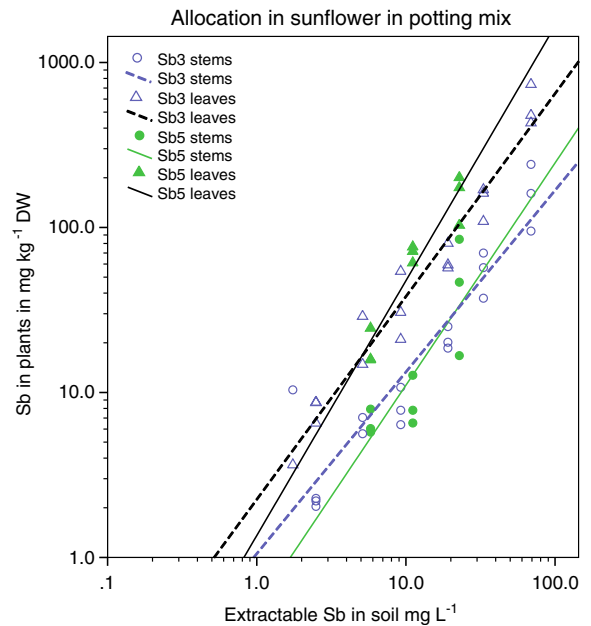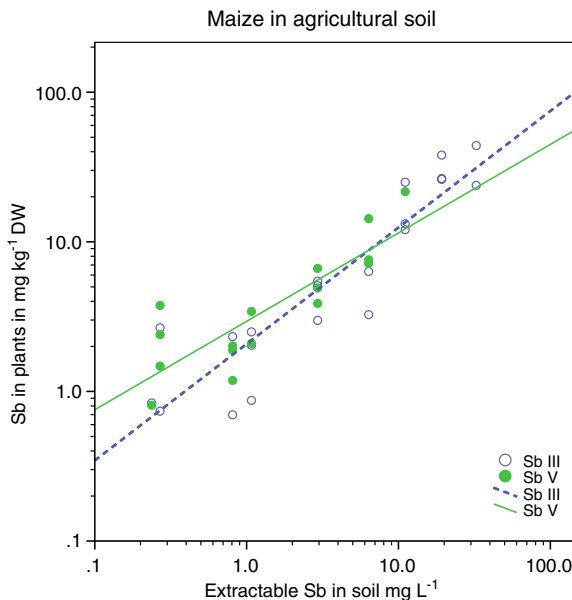

Fig. 7 Accumulation of Sb in leaves and stems of 4-weeks old sunflower plants as a function of the concentration of soluble Sb (mg L^{-1} soil solution) in potting mix to which various amounts of either antimonite (Sb^{III} treatment) or antimonate (Sb^{V} treatment) had been added. *Points* represent experimental data; *lines* were determined by linear regression on the log-transformed data

was also slower in presence of natural Fe oxyhydroxides than with synthetic Fe oxyhydroxides, which can be explained by the increased crystallinity and lower purity of the natural compounds. Oorts et al. (2008) added Sb_2O_3 in suspension to topsoil collected from an uncontaminated agricultural Haplic Luvisol and found that 70% of the Sb in solution was present as Sb^{V} after 2 days. Similarly, we found in a preliminary experiment, in which we added crystalline Sb_2O_3 to the agricultural soil used in our experiments here, that up to 80% of the Sb obtained by KNO_3-extraction was Sb^{V} after 6 h incubation.

Based on the high oxidation rates reported in the literature, almost complete Sb^{III} oxidation was expected in our experiments. However, Sb solubility differed between the two Sb treatments for the potting mix. This difference may be attributed to kinetic limitations in the dissolution of the added Sb_2O_3. In the aforementioned preliminary experiment, we observed that dissolution was the limiting process in the conversion of the added Sb_2O_3 to Sb^{V} in solution. This observation agrees well with the results of Oorts et al. (2008) who found that the concentration of Sb in solution continued to increase

Table 3 Parameter values for the regression equation $\log(c_{plant}) = A*\log(c_{sol}) + \log(B)$ fitted to the experimental relationships between the log-transformed concentrations of soluble soil Sb (c_{sol}, mg L^{-1}) and Sb accumulated in sunflower and maize shoots (c_{plant}, mg kg^{-1} DW) for the two soil substrates and Sb treatments. The numbers in parentheses give the standard errors for A (slope of the regression line) and for the anti-logarithms of the standard errors of log(B), respectively

Substrate	Treatment	H. annuus			Z. mays		
		B	A	R^2	B	A	R^2
Potting mix	Sb III	1.77 (1.26)	1.17 (0.08)	0.93[a]	4.60 (1.36)	0.53 (0.14)	0.50[a]
	Sb V	0.93 (1.40)	1.52 (0.14)	0.95[a]	0.44 (3.58)	0.93 (0.61)	0.37ns
Agricultural soil	Sb III	4.53 (1.16)	0.96 (0.07)	0.89[a]	2.07 (1.17)	0.78 (0.08)	0.84[a]
	Sb V	7.12 (1.16)	0.85 (0.06)	0.88[a]	2.94 (1.14)	0.59 (0.10)	0.73[a]

ns: not significant

[a] : significant at $P<0.01$

linearly with time over 5 weeks after addition of Sb_2O_3, while the final concentration at this time represented only 25% of the added amount of Sb. Thus, it seems likely that also in our experiment dissolution may not have been complete after the 2 weeks of equilibration, and that calculated K_D values thus only represent apparent partition coefficients, which would continue to decrease over time. Oorts et al. (2008) reported K_D values averaging 38 Lkg^{-1} for Sb in soil amended with Sb_2O_3 soil and aged for 5 years. The solubility in the aged soil was similar to that of Sb that had been freshly added to the same soil as $SbCl_3$, indicating that dissolution kinetics was the limiting factor, while sorption occurred rapidly. The (apparent) K_D values calculated in our study are four to six times higher than the average K_D value reported by Oorts et al. (2008) for aged Sb contamination. While these values are still within the range of K_D values reported for Sb in the literature (Cornelis et al. 2006; Tighe et al. 2005), the discrepancy indicates that thermodynamic equilibrium conditions had not been reached. Furthermore, Oorts et al. (2008) observed that the dissolution rate coefficient increased almost three-fold (from 0.005 to 0.014 day^{-1}) when the applied dose of Sb_2O_3 was increased from 0.41 to 81 mmol Sb kg^{-1} soil. The proportionality between soluble Sb concentration and applied Sb dose, however, indicates that the dissolution rate coefficient did not substantially increase with total (nominal) soil Sb concentration in our case.

The fact that the Sb treatment effect on Sb solubility was particularly pronounced for the potting mix can be entirely attributed to higher solubility of antimonate in this substrate, because the soluble soil Sb concentrations did not differ between the two substrates for a given level of Sb_2O_3 application in the Sb^{III} treatments. Buschmann and Sigg (2004) found that oxidation of Sb^{III} can be prevented by complexation with humic matter. However, such an effect cannot explain why there was a difference in Sb solubility between the two substrates in the Sb^V, but not in the Sb^{III} treatment. Higher solubility of antimonate may have been due to the higher organic matter content of the potting mix compared to the agricultural soil, in which the effect of the Sb treatment on Sb solubility was low. Complexation with soluble organic ligands possibly enhanced the solubility of antimonate in the potting mix more than in the agricultural soil. Oorts et al. (2008) found a plateau in Sb solubility at high concentrations of Sb in solution and suggested that this was due to Ca-antimonate precipitation. Here, we found no such plateau and, thus, conclude that such precipitates did not limit Sb^V solubility in our substrates.

While Sb solubility differed little, especially in the agricultural soil, between the Sb^{III} and Sb^V treatments, a clear tendency for higher Sb uptake was observed in the Sb^V treatment in the case of sunflower suggesting that different Sb species were present in solution with different availability for plant uptake and that the concentrations of these Sb species were different in the two Sb treatments. The approximate proportionality between soluble soil Sb and accumulated plant Sb concentrations, extending over two orders of magnitude for sunflower on the agricultural soil, agrees well with previous findings as reviewed by Tschan et al. (2009b), indicating that uptake was dominated by non-selective transport with the transpiration water stream, passing through leaks in the

endodermis or in the case of antimonite also through aquaporins as mentioned already in the introduction. The decrease in Sb accumulation efficiency with increasing Sb concentration that was observed in maize on agricultural soil, but to a slight degree also in sunflower in the Sb^V treatment may reflect to some degree a trend of concentration-dependent Sb speciation; but the differences in these trends between the two plant species show that plant factors also played a role. Unfortunately, the data from the potting mix experiment were too scattered to establish similarly clear trends as for the agricultural soil.

Given that Sb^{III} is generally considered more toxic than Sb^V the lower tolerance of both plants to soluble soil Sb in the Sb^V treatments may be surprising. However, for sunflower, at least, the difference between the two treatments can be explained by the greater Sb accumulation in the Sb^V treatment. For maize, relating toxicity to accumulation did not fully explain the difference in toxicity between the two Sb treatments; it only made it less significant due to increased uncertainty in dose-response curve fitting. A difference in toxicity between the two Sb treatments would mean that Sb speciation in maize could not have been exactly the same or that the toxicity effects on shoot growth were due to interactions between soil Sb and plant roots. In any case, contrary to expectation, the Sb^V treatment was more toxic than or at least as toxic as the Sb^{III} treatment in maize. In sunflower the observed toxicity effects on shoot growth appeared to be related solely to the accumulation of Sb in the shoot tissue. In this case, there either was no difference in toxicity between different Sb species accumulated by the plants or all accumulated Sb was rapidly converted to species of similar toxicity.

The EC_{50} values estimated here from the reponse of shoot growth to soluble Sb in the agricultural soil amended with Sb^{III} ranged between 40 and 50 mg L^{-1} for sunflower and between 16 and 18 mg L^{-1} for maize, depending on the model used to fit the experimental curves. These values are comparable to the EC_{50} values varying around 40 mg L^{-1} (0.34 mmol L^{-1}) that were obtained by Oorts et al. (2008) for lettuce shoot yield. The good agreement between the EC_{50} values obtained with the two models indicates that the estimates were robust.

The dose-response curves obtained here confirm the findings of Oorts et al. (2008) that background soil Sb concentrations are two orders of magnitude lower than typical phytotoxic levels. However, Sb may well occur at phytotoxic concentrations in Sb contaminated soils, in particular in shooting range soils. Our results suggest that even when the predominate form of Sb is antimonate Sb toxicity may still remain a serious risk in Sb contaminated soil.

References

Ainsworth N, Cooke JA, Johnson MS (1990) Distribution of antimony in contaminated grassland 1 vegetation and soils. Environ Pollut 65:65–77

Amereih S, Meisel T, Scholger R, Wegscheider W (2005) Antimony speciation in soil samples along two Austrian motorways by HPLC-ID-ICP-MS. J Environ Monit 7:1200–1206

Asher CJ, Reay PF (1979) Arsenic uptake by barley seedlings. Aust J Plant Physiol 6:459–466

Baes CF, Mesmer RE (eds) (1986) Arsenic, Antimony, Bismuth. In The hydrolysis of cations. pp 366–375. Krieger, Malabar, FL

Baroni F, Boscagli A, Protano G, Riccobono F (2000) Antimony accumulation in *Achillea ageratum*, *Plantago lanceolata* and *Silene vulgaris* growing in an old Sb-mining area. Environ Pollut 109:347–352

Belzile N, Chen YW, Wang ZJ (2001) Oxidation of antimony (III) by amorphous iron and manganese oxyhydroxides. Chem Geol 174:379–387

Blaser P, Pannatier EG, Walthert L (2008) The base saturation in acidified Swiss forest soils on calcareous and non-calcareous parent material. A pH-base saturation anomaly. J Plant Nutr Soil Sci 171:155–162

Blay K (2000) Sorption wässriger Antimon-Spezies an bodenbildende Festphasen und Remobilisierung durch natürliche Komplexbildner. PhD Thesis, Technische Universität München

Bowen HJM (1979) Environmental chemistry of the elements. Academic, London

Buschmann J, Sigg L (2004) Antimony(III) binding to humic substances: influence of pH and type of humic acid. Environ Sci Technol 38:4535

Cornelis G, Van Gerven T, Vandecasteele C (2006) Antimony leaching from uncarbonated and carbonated MSWI bottom ash. J Hazard Mater 137:1284–1292

Filella M, Belzile N, Chen YW (2002) Antimony in the environment: a review focused on natural waters I. Occurence. Earth-Sci Rev 57:125–176

Filella M, Belzile N, Lett MC (2007) Antimony in the environment: a review focused on natural waters. III. Microbiota relevant interactions. Earth-Sci Rev 80:195–217

Gebel T (1997) Arsenic and antimony: comparative approach on mechanistic toxicology. Chem Biol Interact 107:131–144

Gresch M, Wettstein B (2002) Antimon- und Bleibelastung bei Schiessanlagen, Fallbeispiel Eschenbach (SG). Semester Thesis, ETH Zurich

Gulz PA, Gupta SK, Schulin R (2005) Arsenic accumulation of common plants from contaminated soils. Plant Soil 272:337–347

Gurnani N, Sharma A, Talukder G (1994) Effects of antimony on cellular systems in animals—a review. Nucleus 37:71–96

Johnson CA, Moench H, Wersin P, Kugler P, Wenger C (2005) Solubility of antimony and other elements in samples taken from shooting ranges. J Environ Qual 34:248–254

Kabata-Pendias A, Pendias H (1984) Trace elements in soils and plants. CRC Press, Boca Raton

Krachler M, Emons H, Zheng J (2001) Speciation of antimony for the 21st century: promises and pitfalls. Trac-Trends Anal Chem 20:79–90

Leuz AK (2002) Redox chemistry of antimony in aquatic systems: e.g. Lakes. Diploma

Leuz AK, Hug SJ, Wehrli B, Johnson CA (2006a) Iron-mediated oxidation of antimony(III) by oxygen and hydrogen peroxide compared to arsenic(III) oxidation. Environ Sci Technol 40:2565–2571

Leuz AK, Monch H, Johnson CA (2006b) Sorption of Sb(III) and Sb(V) to goethite: influence on Sb(III) oxidation and mobilization. Environ Sci Technol 40:7277–7282

Oorts K, Smolders E, Degryse F, Buekers J, Gasco G, Cornelis G, Mertens J (2008) Solubility and toxicity of antimony trioxide (Sb$_2$O$_3$) in soil. Environ Sci Technol 42:4378–4383

Pratas J, Prasad MNV, Freitas H, Conde L (2005) Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation. J Geochem Explor 85:99–107

Rooney CP, McLaren RG, Cresswell RJ (1999) Distribution and phytoavailability of lead in a soil contaminated with lead shot. Water Air Soil Pollut 116:535–548

Sachs L (2004) Angewandte Statistik. Springer, Berlin

SPSS for Windows, Rel. 17.0.0. (2008) Chicago: SPSS Inc

Tighe M, Lockwood P, Wilson S (2005) Adsorption of antimony(v) by floodplain soils, amorphous iron(III) hydroxide and humic acid. J Environ Monit 7:1177–1185

Tschan M, Robinson B, Schulin R (2008) Antimony uptake by Zea mays (L.) and Helianthus annuus (L.) from nutrient solution. Environ Geochem Health 30:187–191

Tschan M, Robinson BH, Nodari M, Schulin R (2009a) Antimony uptake by different plant species from nutrient solution, agar and soil. Environ Chem 6:144–152

Tschan M, Robinson BH, Schulin R (2009b) Antimony in the soil-plant system—a review. Environ Chem 6:106–115

Woolson EA, Axley JH, Kearney PC (1973) Chemistry and phytotoxicity of arsenic in soils. 2. Effects of time and phosphorus. Soil Sci Soc Am J 37:254–259